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GENE REGULATION IN PSORIATIC KERATINOCYTES: MICRORNAs AND CYTOKINE RESPONSES

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Gene regulation in psoriatic keratinocytes: miRNAs and cytokine responses

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In loving memory of my father

"Once more into the fray, into the last good fight I'll ever know.

Live and die on this day, live and die on this day."

"The Grey"

ABSTRACT

Psoriasis is the most common chronic skin inflammation in adults which affects 2-4% of the world population. Keratinocytes are the key structural cells in epidermis which hyper-proliferate and undergo aberrant differentiation in psoriasis. Keratinocyte-derived cytokines and chemokines are essential in maintaining the vicious auto-inflammatory loop in psoriasis. MicroRNAs (miRNAs) are small RNAs (22-25 nucleotides) which regulates gene expression at the post-transcriptional level. In this thesis, we explored the functional role of miRNAs/ cytokine networks in psoriasis.

Paper I: A strong protective association of SNP (rs2910164-CC) of miR-146a to early onset of psoriasis was observed in *HLA-C*06*-negative patients. In addition, miR-146a deletion in mice led to earlier onset, more severe inflammation and impaired healing of imiquimod-induced psoriasis-like skin inflammation. Therapeutically, miR-146a mimic injection prevented psoriasiform skin-inflammation in WT mice, suggesting an anti-inflammatory role of miR-146a.

Paper II: Here we explored the landscape of miRNAs in psoriatic keratinocytes. We identified several miRNAs, which were not identified in psoriasis before and their expression was altered in keratinocyte. In addition, upregulation of miR-1307-3p and human-specific miR-941 was validated with qRT-PCR.

Paper III: MiR-149 was found to be downregulated in psoriatic keratinocytes compared to keratinocytes from non-lesional or healthy skin. *In vitro* studies revealed that the downregulation of miR-149 is mediated by IFN-γ/JAK/STAT axis. Functionally, miR-149 targeted the inflammatory and JAK/STAT pathway genes in resting as well as in IFN-γ treated keratinocytes. Moreover, miR-149 inhibition potentiated IFN-γ responses in keratinocytes.

Paper IV: Here we outlined a new mode of action of tofactinib, a JAK inhibitor with effects on psoriasis in clinical trials. We showed that JAK proteins that are targets of tofacitinib are expressed by keratinocytes and tofacitinib had a robust impact on IL-22- regulated transcriptome in keratinocytes. Our results imply that effects of tofacitinib are not just limited to T-cells as previously thought but can also be observed in keratinocytes.

LIST OF PUBLICATION

I. **MicroRNA-146a suppresses IL-17–mediated skin inflammation and is genetically associated with psoriasis**
Ankit Srivastava, Pernilla Nikamo, Warangkana Lohcharoenkal, Dongqing Li, Florian Meisgen, Ning Xu Landén, Mona Ståhle, Andor Pivarcsi and Enikö Sonkoly

J Allergy Clin Immunol, 2016 Aug; 139(2): 550-560

II. **Next generation sequencing identifies the keratinocyte-specific miRNA signature of psoriasis**
Ankit Srivastava, Florian Meisgen, Lorenzo Pasquali, Sara Munkhammar, Ping Xia, Mona Ståhle, Ning Xu Landén, Andor Pivarcsi and Enikö Sonkoly

Submitted

III. **miR-149 is downregulated in psoriasis and regulates the JAK-STAT pathway in keratinocytes**
Ankit Srivastava, Florian Meisgen, Mona Ståhle, Andor Pivarcsi and Enikö Sonkoly

Manuscript

IV. **Tofacitinib represses the Janus Kinase-Signal Transducer and Activators of Transcription signalling pathway in keratinocytes**
Ankit Srivastava, Mona Ståhle, Andor Pivarcsi and Enikö Sonkoly

Acta Derm Venereol 2018 May; 98: 772–775

PUBLICATION NOT INCLUDED IN THIS THESIS

Identification of chronological and photoageing-associated microRNAs in human skin
Ankit Srivastava, Magnus Karlsson, Claire Marionnet, Françoise Bernerd, Audrey Gueniche, Charles E. l. Rawadi, Mona Ståhle, Enikö Sonkoly, Lionel Breton and Andor Pivarcsi

Scientific Reports 2018 Aug; 8 (12990) 1-11

The keratinocyte transcriptome in psoriasis: pathways related to immune responses, cell cycle and keratinization
Lorenzo Pasquali, Ankit Srivastava, Florian Meisgen, Kunal Das Mahapatra, Ping Xia, Ning Xu Landén, Andor Pivarcsi and Enikö Sonkoly

Submitted

CONTENTS

LIST OF ABBREVIATIONS

3’-UTR	Three prime untranslated region
AMP	Antimicrobial peptide
CCL	CC chemokine ligand
CCR	CC chemokine receptor
CD	Cluster of differentiation
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
ELISA	Enzyme-linked immunosorbent assay
FFPE	Formalin-Fixed, Paraffin-Embedded
GSEA	Gene set enrichment analysis
GWAS	Genome-wide association study
HKGS	Human keratinocyte growth supplement
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IKK	IκB-kinase
IL	Interlukin
JAK	Janus kinase
MAPK	Mitogen-activated protein kinase
miRNA	microRNA
MMP	Matrix metalloproteinases
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NKT cells	Natural killer T cells
NLR	Nucleotide-binding domain, leucine-rich repeat
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
pDCs	Plasmacytoid dendritic cells

Pri-miR	Primary microRNA
PRR	Pattern recognition receptor
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RIN	RNA integrity number
RISC	RNA-induced silencing complex
SALT	Skin-associated lymphoid tissue
SCID	Severe combined immunodeficiency
siRNA	Small interfering RNA
SNP	Single nucleotide polymorphism
SOCS-3	Suppressor of cytokine signaling 3
STAT	Signal transducer and activator of transcription
T_H	T helper cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
TRAF	TNF receptor-associated factor 6
UV	Ultraviolet

1 BACKGROUND

Being the largest organ of the body, skin is the first line of defence against pathogens, heat, chemical and toxic insults (Di Meglio et al., 2011, Nestle et al., 2009a). Often, devising an adequate immunological response to the insult is a great challenge; as an insufficient response can result in life threatening infections or tumour formation while an overwhelming response can result in chronic inflammation or autoimmune diseases (Di Meglio et al., 2011, Nestle et al., 2009a). In this thesis we made an attempt to understand the pathophysiological mechanisms of an inflammatory skin disease- psoriasis, focusing on miRNA-mediated gene regulation and cytokine signalling in keratinocytes.

1.1 The skin

The skin is constantly exposed to external provocations and microorganisms (Di Meglio et al., 2011, Nestle et al., 2009a, Simpson et al., 2011) and forms the primary interface between the environment and the organism by creating physical, chemical and immunological barrier (Di Meglio et al., 2011, Simpson et al., 2011). Apart from synthesising vitamins (vitamin D3) (Holick et al., 1987) and hormones (sex hormones and insulin-like growth factor-binding proteins) (Zouboulis, 2000) skin also restricts water loss, resist mechanical stress, protects from potential assault by foreign organisms or toxic substances and act as a sensory organ (Di Meglio et al., 2011, Simpson et al., 2011). Human skin consist of an outer layer (epidermis), and inner layer (dermis) and hypodermis (*Figure 1*).

1.1.1 Epidermis

The epidermal compartment is majorly composed of keratinocytes (>90%), however melanocytes, immune cells (Langerhans cells and T lymphocytes) and Merkel cells also reside in the epidermis (*Figure 1*) (Di Meglio et al., 2011, Nestle et al., 2009a). From the innermost

layer to the outermost layer, the epidermis is divided into the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum (*Figure 1*).

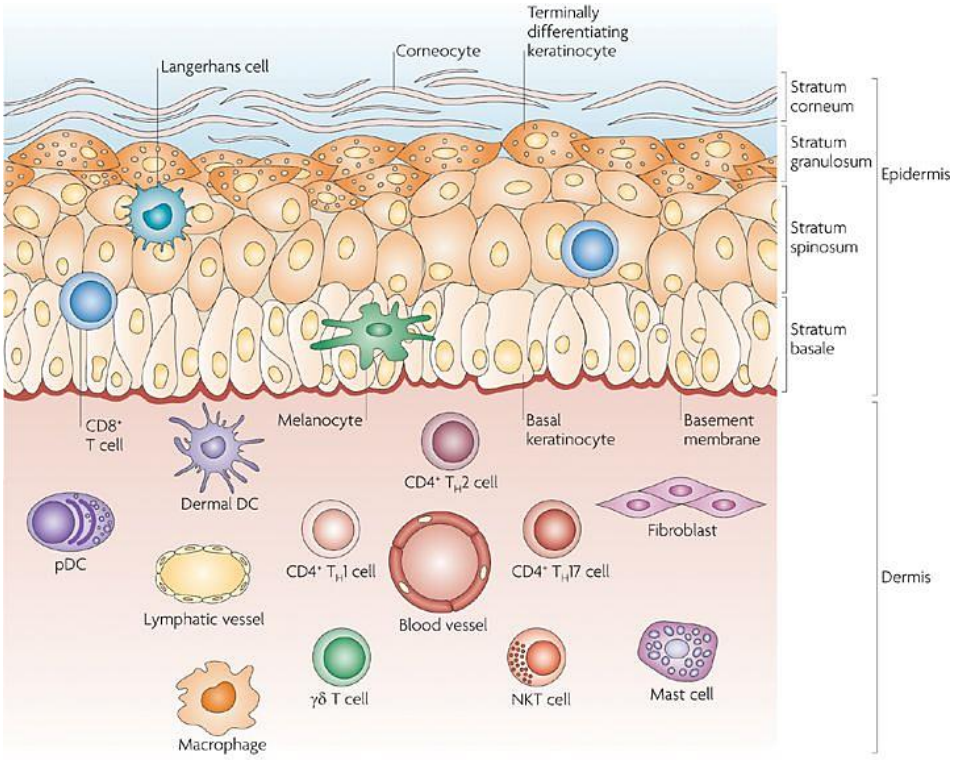


Figure 1: Skin anatomy and effector cells. Reproduced with permission from Nestle et al., 2009. Copyright Springer Nature.

Keratinocytes start to differentiate as they leave the stratum basale and finally die and ultimately shed off from the body at stratum corneum (*Figure 1*) (Simpson et al., 2011). In the stratum basale, keratinocytes (stem-like-cells) proliferate and divide (*Figure 1*) (Blanpain and Fuchs, 2009, Simpson et al., 2011). In the stratum spinosum, keratinocytes lose their mitotic activity to increase in size and form intracellular connections via gap junctions and desmosomes. In the stratum granulosum, keratinocytes starts to get flattened and a water impermeable cornified envelop underlying plasma membrane is formed (Di Meglio et al., 2011, Simpson et al., 2011). In the stratum corneum, keratinocytes become compactly crosslinked to form ultimate skin barrier (*Figure 1*). The desquamation of the cornified layer of epidermis maintains the division of keratinocytes at the basal layer (Simpson et al., 2011). The differentiation of keratinocytes is a tightly regulated process maintained by signalling

pathways, transcription factors and epigenetic regulation (Botchkarev, 2015). Keratins, K5 and K14 which forms keratin intermediate filaments are the two essential proteins involved in the proliferation of basal keratinocytes (Candi et al., 2005, Fuchs and Raghavan, 2002, Mack et al., 2005). Progression of differentiation in keratinocytes sequentially induce the expression of differentiation associated keratins. As the basal keratinocytes move upward the keratin intermediate filament structures formed by K5 and K14 are replaced by K1 and K10 filaments. K1 and K10 are the markers of early differentiation and start to get expressed in the stratum granulosum at the initial stage of cornification (Candi et al., 2005, Fuchs and Raghavan, 2002, Mack et al., 2005). At the later stage of cornification filaggrin aggregates keratin filaments into compact bundles (Candi et al., 2005, Fuchs and Raghavan, 2002, Mack et al., 2005). Another two important structural protein loricrin and involucrin reinforce the cornified layer just under the plasma membrane (Candi et al., 2005, Fuchs and Raghavan, 2002, Mack et al., 2005).

Langerhans cells are skin-specific antigen presenting cells present in the epidermis (*Figure 1*). Dendrites of the Langerhans cells are in contact with the skin surface, continuously sensing for foreign antigens (Di Meglio et al., 2011, Nestle et al., 2009a). Melanocytes are another cell type of epidermis which produce melanin and protects keratinocytes against UV-radiation-induced DNA-damage (*Figure 1*) (Di Meglio et al., 2011, Nestle et al., 2009a). In addition, nerve-ending Merkel cells are also present in the epidermis which facilitate light-touch to distinguish between different shapes and texture (Di Meglio et al., 2011, Nestle et al., 2009a).

1.1.2 Dermis

Underneath the basement membrane, networks of loose and dense collagen and elastin fibers constitute upper papillary (stratum papillare) and lower reticular (stratum reticulare) layer of dermis. The primary role of dermis is to provide stability and flexibility to the skin and nutrients to epidermis. Fibroblasts and fibrocytes are the two resident cells maintaining the extracellular matrix which provide structural support for blood vessels, sweat glands, sebaceous glands and

hair follicles (*Figure 1*) (Di Meglio et al., 2011, Nestle et al., 2009a, Pasparakis et al., 2014). The dermis also hosts immune cells such as dermal dendritic cells, T cells, natural killer cells, B cells, mast cells and macrophages (*Figure 1*) (Di Meglio et al., 2011, Nestle et al., 2009a, Pasparakis et al., 2014).

1.1.3 Hypodermis

The hypodermis is the lowermost layer of skin composed of loose connective tissue and fat lobules rich in fibroblast, adipose cells, macrophages and monocytes. The prime function of hypodermis is the fat storage and maintenance of body temperature by heat insulation. Adipose tissue may contribute to immune response either by direct effect on resident immune cells or by modulating immune function in endocrine or paracrine fashion (Desruisseaux et al., 2007, Pasparakis et al., 2014).

1.1.4 Immune sentinels in the skin

In addition to its non-immune functions, the skin serves as immune organ to defend internal tissues from foreign agents, while also maintaining homeostasis and preventing excessive inflammation. In 1983, skin was coined as skin-associated lymphoid tissue (SALT) due to the immune cell trafficking from draining lymph nodes to skin (Streilein, 1983). Since then vast amount of research has been performed to decipher the role of different skin resident cells in mediating immune response. The skin sentinels mediates both innate and adaptive immune responses.

Keratinocytes are the first line of immune surveillance (*Figure 2*) and recognizes pathogens through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) which are continuously scanning for pathogen-associated molecular patterns (PAMPs) (Di Meglio et al., 2011). Keratinocytes express several TLRs either on cell surface such as TLR1 (ligand-

lipopeptides), TLR2 (ligands- lipoprotein, peptidoglycans and zymosan), TLR4 (ligand- lipopolysaccharide), TLR5 (ligand- Flagellin) and TLR6 (ligand- lipopeptides and lipteichoic acid) or on endosomes such as TLR9 (ligand- (unmethylated) CpG-containing DNA) and TLR3 (ligand- double stranded RNA) (Lebre et al., 2007, Pivarcsi et al., 2003). In addition, keratinocytes express nucleotide-binding domain, leucine-rich repeat (NLR) genes which also recognize PAMPs and danger-associated molecular patterns (DAMPs) (Martinon et al., 2009). Ligand binding to these receptors leads to inflammasome-mediated activation of pro-inflammatory signalling cascade (Martinon et al., 2009).

The nuclear factor-κB (NF-κB) signalling pathway is one of the pivotal pro-inflammatory signalling pathway activated in keratinocytes upon pathogen recognition and associated with chemokine and cytokine production in response to TNF-α, IL-1 and TLRs (Goldminz et al., 2013, Pivarcsi et al., 2003). In the normal resting cells, NF-κB (p65) is bound to its inhibitory proteins known as inhibitor of kappa B (IκBs) and confined to cytoplasm (Fuchs and Raghavan, 2002, Goldminz et al., 2013, Mack et al., 2005). In the active state of signalling p65 translocate to the nucleus to exert its function. NF-κB pathway targets a variety of genes influencing biological processes such as innate and adaptive immunity, inflammation and response against stress (Fuchs and Raghavan, 2002, Goldminz et al., 2013, Mack et al., 2005).

Upon pathogen engagement keratinocytes secrete anti-microbial peptides such as defensins, LL-37 and S100s (Di Meglio et al., 2011, Gilliet and Lande, 2008, Lai and Gallo, 2009, Lowes et al., 2014). Keratinocytes produce pro-inflammatory cytokines such as IL-1β and IL-18, IL-6 and TNF in response to external injuries such as infections and trauma (Albanesi et al., 2005, Nestle et al., 2009a). To amplify skin inflammation, keratinocytes also secrete chemokines to enhance immune cell traffic to the skin by chemotaxis (Albanesi et al., 2005, Di Meglio et al., 2011, Kim and Krueger, 2015, Lowes et al., 2014). Chemokines bind to G-protein coupled receptors (Nedoszytko et al., 2014, Turner et al., 2014). The expression of chemokines, their receptor and the adhesion molecule contribute to the homing and migration of leukocytes

(Nedoszytko et al., 2014, Turner et al., 2014). For example CXCL1, CXCL2 (ligands of CXCR2), CXCL8/IL-8 (ligand of CXCR1, CXCR2) are chemotactic for neutrophils granulocytes, which express their receptors and can stimulate their migration to the skin (Albanesi et al., 2005, Nedoszytko et al., 2014, Turner et al., 2014). Similarly, CXCL9, 10 and 11 (receptor-CXCR3) are chemotactic for T_H1 cells (Nedoszytko et al., 2014, Turner et al., 2014). CCL20 (the ligand of CCR6) is another key pro-inflammatory chemokine secreted by keratinocytes which chemotactically attracts T_H17, T_H22 and dendritic cells to the skin (Nedoszytko et al., 2014, Turner et al., 2014).

Dendritic cells (DCs) are important in the immuno-surveillance of the skin (*Figure 2*) and are specialized in antigen presentation. DCs in skin migrates to lymph nodes where they present the antigen and drive the activation and differentiation of T cells. DCs serve as an essential link between innate and adaptive immunity. In addition, DCs secrete pro-inflammatory mediators (inflammatory DCs) (Bieber, 2007, Lowes et al., 2005), produce IFNs (plasmacytoid DCs) (Gilliet et al., 2008, Nestle et al., 2009a). Antigen presenting Langerhans cells are another subset of DCs which reside in epidermis and among the first line of immune sentinels sensing the foreign particles (Nestle et al., 2009a, Pasparakis et al., 2014). Moreover, migratory dermal DCs are potent regulator of T-cell proliferation in draining lymph nodes (Nestle et al., 2009a, Pasparakis et al., 2014).

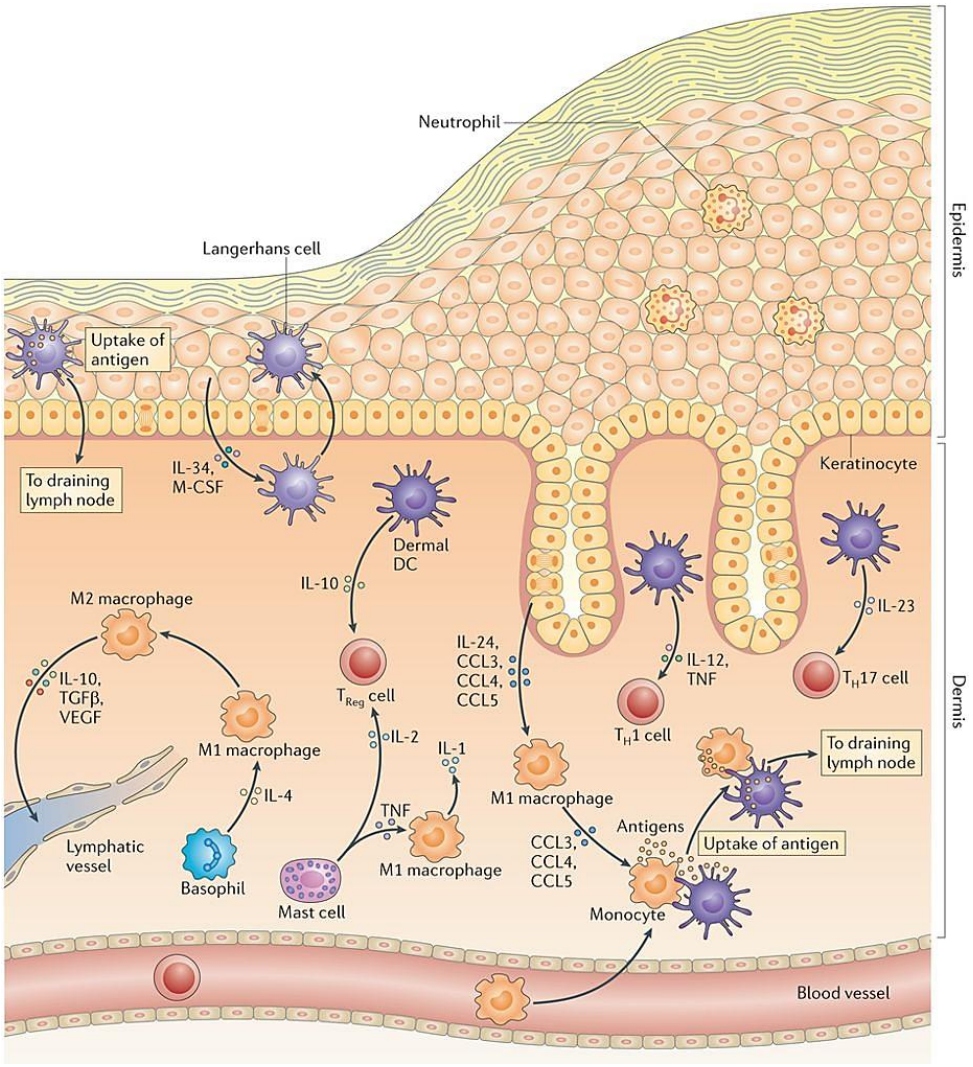


Figure 2: Skin immune sentinels. Reproduced with permission from Nestle et al., 2014. Copyright Springer Nature.

Both **cytotoxic ($CD8^+$)** and **helper ($CD4^+$)** T-cells are present in the skin and mediate acquired immune response (*Figure 2*) (Di Meglio et al., 2011, Nestle et al., 2009a, Pasparakis et al., 2014). Normal skin harbours twice the amount of T-cell present in circulation (Clark et al., 2006). **T_H1, T_H2, T_H17, T_H22** and **regulatory T** cells are the main subsets of $CD4^+$ T-cell mediating the immune response to different pathological insults. T_H1 cells mediates anti-viral and anti-tumour response of the skin, while T_H17 cells mediate anti-bacterial and anti-fungal responses. In addition, T_H2 cells facilitates immune response to different allergens and parasites (Di Meglio et al., 2011, Nestle et al., 2009a, Pasparakis et al., 2014).

Neutrophils are a part of innate immunity and another source of antimicrobial peptides and are rapidly recruited to skin upon bacterial infections in response to chemokines secreted by keratinocytes (*Figure 2*) (Abtin et al., 2014, Albanesi et al., 2005).

Mast cells, macrophages, NKT cells and innate lymphoid cells are the other immune sentinels either present in skin or in contact via trafficking through circulation to mediate immune response towards internal or external immune triggers (*Figure 2*) (Di Meglio et al., 2011, Jenkins et al., 2011, Nestle et al., 2009a, Pasparakis et al., 2014, St John et al., 2011, Villanova et al., 2014).

Communication among the skin resident cells as well as the trafficking immune cells is critical to maintain skin homeostasis and to provide an appropriate response to the invading foreign triggers. However, failure in the crosstalk among skin resident and trafficking immune cells and their responses could result in chronic auto-inflammatory diseases, such as psoriasis.

1.2 Psoriasis

Psoriasis is the most common immune-mediated inflammatory skin disease in adults, affecting 2-3% of world population with higher prevalence in north American, Canadian and European population than African and Asian populations (Naldi, 2004). It is a lifelong chronic disease with spontaneous remission and exacerbations (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). While the majority of patients have mild disease, approximately one third of the psoriasis patients have moderate to severe psoriasis with more than 10% of the body covered with psoriatic lesions (Lowes et al., 2014). Psoriasis impairs the quality of life substantially, which is comparable to systemic lifelong disease such as diabetes (Rapp et al., 1999). While psoriasis was previously considered as merely a skin disease, today it is considered a systemic disease with several comorbidities such as psoriatic arthritis, inflammatory bowel disease, depression and metabolic syndrome such as obesity, hypertension, and insulin resistance (Javitz et al., 2002, Lowes et al., 2014, Sohn et al., 2006, Yu et al., 2009).

Plaque psoriasis or psoriasis vulgaris is the most prevalent type of the disease, typically manifested as red plaques with demarcated silvery-white dry scales located on elbows, knees, scalp and face (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). Apart from the plaque psoriasis there are other less common types of psoriasis such as guttate, inverse and pustular psoriasis.

Histologically, psoriasis lesions are marked by thickened epidermis with significant structural alterations such as parakeratosis (retention of nuclei within the cells of the stratum corneum), acanthosis (thickening of epidermis), papillomatosis (rete ridge formation), hypogranulosis (loss of granular layer) and massive infiltration of immune cells (DCs, T_H1, T_H17, and T_H22 cells) within the papillary dermis and epidermis and neutrophil microabscesses (*Figure 3*) (Kim and Krueger, 2015, Lowes et al., 2007, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012).

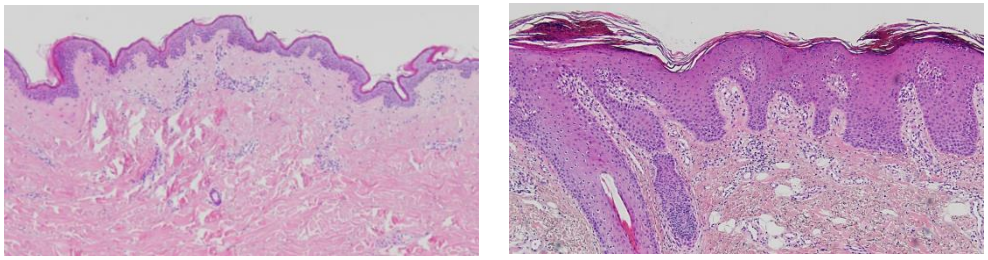


Figure 3: Histology of healthy (left) and psoriasis (right) skin. Courtesy of Dr. Britta Krynitz (Department of pathology, Karolinska Institutet).

1.2.1 Genetics of psoriasis

Population and family-based studies in the 1960s revealed a higher incidence of psoriasis among relatives of the psoriasis patients (60-90% heritability) than the general population, indicating a genetic predisposition to the disease (Farber and Nall, 1974, Girolomoni et al., 2015, Nestle et al., 2009b, Perera et al., 2012). Later in 1970s twin studies from Denmark, USA and Austria presented a concrete evidence of genetic predisposition in psoriasis, as monozygotic twin concordance rate was higher (35-73%) than the dizygotic twins (12-20%) (Brandrup et al., 1978, Duffy et al., 1993, Farber et al., 1974). Nevertheless, less than 100% concordance rate in monozygotic twins suggested a multifactorial aetiology of the disease, with the involvement of environmental factors in addition to the genetic contribution (Elder et al., 1994, Farber and Nall, 1974, Girolomoni et al., 2015, Nestle et al., 2009b, Perera et al., 2012, Watson et al., 1972). Since then at least 36 genomic susceptibility loci have been identified and associated with psoriasis (Ray-Jones et al., 2016, Tsoi et al., 2012, Tsoi et al., 2017). The psoriasis susceptibility-1 (PSORS1) region was first identified as a potential susceptibility locus in 1970s. PSORS1 corresponds to major histocompatibility complex (MHC) and the key associated allele in class I MHC molecule HLA-C is HLA-Cw*0602 which is present in 40-60% of psoriasis patients and confers a 20-fold higher risk of psoriasis, however, approximately only 1 out of 10 HLA-Cw*0602 carriers develop psoriasis. (Elder, 2017, Perera et al., 2012, Russell et al., 1972, Tiilikainen et al., 1980). Over the years, genome wide association studies (GWAS) led to advancement in psoriasis genetics and identified genes involve in the psoriasis

pathogenesis and progression and most of them belong to adaptive immunity (such as IL-23/Th-17 axis) innate immunity (NF-κB pathway genes), and keratinocytes (such as genes regulating epidermal differentiation) (Kim and Krueger, 2015, Lowes et al., 2014, Ray-Jones et al., 2016).

Despite the discovery of a large number of susceptibility genes in psoriasis, there is still a missing heritability which cannot be explained by the combination of all the known factors (Pivarcsi et al., 2014). Genome wide association studies focuses on the protein coding genes and the noncoding regulators such as miRNAs have not been studied in vast details (Pivarcsi et al., 2014).

1.2.2 Immunopathogenesis of psoriasis

Environmental factors such as injury, trauma, stress, alcohol, smoking and infections may trigger psoriasis in genetically predisposed individuals (Perera et al., 2012). Recently studies have suggested a role of autoantigens in psoriasis such as i) cathelicidin (LL37), a keratinocyte and neutrophils-derived antimicrobial peptide which meditates DC activation leading to T cell expansion and activation (Lande et al., 2014), ii) cytosolic phospholipase A2 lipid antigen, (PLA2G4D), a keratinocyte and mast cell-derived lipid antigen recognized by CD1a- restricted T cells, thus inducing IL-17A and IL-22 production (Cheung et al., 2016), iii) HLA-C*06:02-restricted melanocyte-derived antigen, a disintegrin and metalloprotease domain containing thrombospondin type 1 motif- like 5 (ADAMTSL5) which activates T_H17 cells (Arakawa et al., 2015). The role of autoantigen is still a budding concept in psoriasis and more clinical evidences are required to establish the pathogenic role of one or more autoantigens.

The current model of psoriasis initiation banks on a key finding which demonstrated that psoriatic skin extract was able to activate plasmacytoid dendritic cells (pDCs) mediated production of IFNs via cathelicidine antimicrobial peptide LL37/DNA complex (Lande et al.,

2014, Nestle et al., 2009a). According to this model stressed keratinocytes release self-DNA and RNA, which binds to LL37, and activates pDCs in TLR9- or TLR7-dependent manner to produce interferon-α (IFN-α) (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). More recently, it has been shown that LL37/RNA complex can also induce myeloid dendritic cells through TLR8 (Lowes et al., 2014). Interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF-α) which are produced by keratinocytes along with pDCs derived IFN-α activates dermal DCs (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). Activated dermal dendritic cells (DCs) migrate to the skin draining lymph nodes and present an unknown antigen to encourage naive T-cells to differentiate in to T_H1 and T_H17 cells (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). DC-derived IL-12 and IL-23 are the drivers of this differentiation and are required for expansion and survival of T_H1 and T_H17 cells respectively (*Figure 4*) (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012).

1.2.2.1 T_{Helper} cells: help gone wrong

T cells are present in high number in psoriasis (Zaba et al., 2010) and the pivotal role of T cells was first evidenced by the potential of chimeric anti-CD4 antibody to treat psoriasis (Prinz et al., 1991). These findings were further corroborated by the mouse model of psoriasis (non-lesional skin transplantation on to severe combined immune-deficient mice), where psoriasis developed after injection of autologous activated CD4+ T cells (Boyman et al., 2004). For many years psoriasis has been thought to be a **T_H1**-mediated disease and even after emergence of a central role of T_H17 cells in mediating psoriasis pathogenesis, T_H1 cells still remain an accessory to the disease (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). T_H1 cells are present in high numbers in psoriasis and T_H1-mediated anti-viral responses are enriched in psoriasis skin (Kagami et al., 2010, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012, Szabo et al., 1998, Uyemura et al., 1993). T_H1 cells express CXC-chemokine receptor 3 (CXCR3) and CC-chemokine receptor 4 (CCR4) which respond to chemo-attractants such as

CXCL9, 10, 11 released by keratinocytes and myeloid cells and migrate to psoriatic lesions. IFN-γ and TNF-α are the two main cytokines mediating T_H1 responses in psoriasis (*Figure 4*) (Lowes et al., 2007, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012).

Recent development in the psoriasis research have revealed a central role for **T_H17** cells (Lowes et al., 2008, Wilson et al., 2007), in the disease pathogenesis and progression. There is a higher magnitude of infiltrating T_H17 cells in psoriasis lesions (Chan et al., 2006, Kagami et al., 2010, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012, Wilson et al., 2007). T_H17 cells differ from classical T_H1 and T_H2 by their ability to produce IL-17A along with IL-17F and IL-22 (Martin et al., 2013). The genome wide association studies identified that several genes associated with psoriasis belong to the IL-23 and IL-17 signalling pathway (Lowes et al., 2014, Martin et al., 2013, Nair et al., 2009, Nestle et al., 2009a, Nestle et al., 2009b, Perera et al., 2012, Veal et al., 2001). Infiltrating T_H17 cells express CCR4 and CCR6 and attracted to the site of lesion by CCL20 secreted by keratinocytes and DCs (*Figure 4*) (Albanesi et al., 2001, Harper et al., 2009, Martin et al., 2013). A recent study have shown that a subset of T_H17 cells can also produce IFN-γ together with IL-17A (Cheuk et al., 2017, Zielinski et al., 2012).

Infiltrating **T_H22** cells producing IL-22 are also enriched in psoriasis (Kagami et al., 2010, Nestle et al., 2009b, Perera et al., 2012, Wolk et al., 2006). T_H22 cell express skin homing markers CCR4, CCR6 and CCR10 and respond to CCL20 secreted by keratinocytes (*Figure 4*) (Perera et al., 2012, Wolk et al., 2006).

1.2.2.2 Keratinocytes: amplifiers of inflammation

Keratinocytes are key structural cells in maintaining psoriasis hallmarks. In psoriasis, keratinocytes hyper-proliferate, undergo an aberrant differentiation program and produce pro-inflammatory cytokines and chemokines (*Figure 4*). Keratinocyte-specific genes such as late cornified envelope (LCE) are present in psoriasis genetic susceptibility locus (Capon et al.,

1999a, Capon et al., 1999b, de Cid et al., 2009, Perera et al., 2012, Zhang et al., 2009) and there is an enrichment of keratinocytes anti-microbial (IL-17/IL-22) and anti-viral (IFN-γ) response gene sets in psoriasis transcriptome (Lowes et al., 2014, Perera et al., 2012)

The role of keratinocytes in psoriasis is further supported by mouse models in which keratinocyte-specific genetic alterations have resulted in psoriasis-like symptoms. For example, mice with targeted deletion of JUN, JUNB or SOCS3 in keratinocytes spontaneously developed inflammation in the skin (Uto-Konomi et al., 2012, Zenz et al., 2005) and mice with constitutive activation of signal transducer and activator of transcription 3 (STAT3) or overexpression of IL-17C in keratinocytes developed psoriasis like inflammation (Johnston et al., 2013, Sano et al., 2005).

Furthermore, keratinocytes express receptors for IL-17A (IL-17R), IFN-γ (IFN-γR) and IL-22 (IL10R/IL22R) in psoriasis (Lowes et al., 2014, Martin et al., 2013, Nestle et al., 2009b, Perera et al., 2012). Keratinocytes are the major source of IL-1β, a cytokine required for naïve T cell differentiation to T_H17/T_H1 cells and their activation (Debets et al., 1997, Kim and Krueger, 2015, Lowes et al., 2014, Murphy et al., 2000, Nestle et al., 2009b, Perera et al., 2012). The cross-talk of keratinocytes with T_H17 cells via IL-17A and CCL20 and with T_H1 cells via IFN-γ and CXCL9, 10, 11 is important in disease progression. Upon stimulation with IL-17A keratinocytes produce CCL20 and IL-8/CXCL8 (Albanesi et al., 2005, Harper et al., 2009, Kim and Krueger, 2015, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). CCL20 is a chemokine which recruits T_H17 and myeloid DCs to the site of psoriatic lesions (Kim and Krueger, 2015, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012) while IL-8 is a chemoattractant for neutrophils and recruits them to the site of lesions (Albanesi et al., 2005, Albanesi et al., 2001, Kim and Krueger, 2015, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). Furthermore, IL-17A induces keratinocytes to produce antimicrobial peptides (AMPs)-defensins and S100As proteins (S100A7, A8, and A9) to induce and enhance inflammatory responses (Kim and Krueger, 2015, Lowes et al., 2014, Nestle et al., 2009b,

Nograles et al., 2008, Perera et al., 2012). IFN-γ stimulates keratinocytes to produce inflammatory cytokines- IL-6, anti-viral proteins and chemokines CXCL9, 10 and 11 which recruits T_H1 cells (Albanesi et al., 2005, Albanesi et al., 2001, Kim and Krueger, 2015, Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012). In addition, IL-22 synergises with IL-17A and enhances production of matrix metalloproteinases (MMPs) and AMPs from keratinocytes to induce rete ridge formation in psoriasis (Wolk et al., 2006). In summary, keratinocytes are the key element in the auto-inflammatory loop of psoriasis and act as an amplifier of cellular inflammatory signals.

1.2.2.3 Cytokine networks in psoriasis

Cytokines are class of small proteins involved in cell signalling and mediates autocrine, paracrine and endocrine signalling as immunomodulating agents. Psoriasis-associated cytokine networks are discussed in the following section.

IFN-γ belongs to type II family of IFNs and mainly produced by T_H1, T_H17, T_C, NK and NKT cells (*Figure 4*) (Cheuk et al., 2017, Hassan-Zahraee et al., 1998, Perera et al., 2012, Zielinski et al., 2012). IFN-γ is important for myeloid dendritic cells to produces IL-12 and IL-23, thus regulating the T_H1 and T_H17 cell differentiation. Keratinocytes harbour IFN-γ receptor, which upon binding to IFN-γ initiates inflammatory and anti-viral responses of keratinocytes via the JAK1-2/STAT1 signalling cascade (Ramana et al., 2002). IFN-γ-induced chemokines and cytokines such as CXCL 9, 10, 11 and IL-6 has been shown to be important in psoriasis by regulating T-cell trafficking and inflammation (Kanda et al., 2007). Recently a study demonstrated that a single intradermal injection of IFN-γ resulted in inflammation in both non-lesional psoriatic and healthy skin (Johnson-Huang et al., 2012). Elevated expression of IFN-γ mRNA has been shown in psoriasis skin lesions along with increased levels of IFN-γ in serum of psoriasis patients (Abdallah et al., 2009, Lowes et al., 2014). The important role of IFN-γ in

psoriasis is further corroborated by transcriptomic analysis of psoriasis lesional skin, which revealed enrichment of IFN-γ/STAT1 related genes among the differentially expressed genes (Swindell et al., 2013). Furthermore, STAT1 expression is increased in psoriasis skin along with increased phosphorylation of p-STAT1 (Hald et al., 2013). Therapeutically, anti-IFN-γ (HuZAF) has been shown to have some efficacy in treating psoriasis patients (Harden et al., 2015).

TNF-α regulates cellular processes such as inflammation, differentiation, proliferation, cell growth, survival and apoptosis (Gaur and Aggarwal, 2003, Locksley et al., 2001). It is mainly produced by mast cells, DCs, T_H1 and T_H17 cells (Nestle et al., 2009b, Perera et al., 2012). TNF-α play an important role in the activation of DCs in psoriasis and anti-TNFs (such as etanercept, adalimumab and infliximab) have shown great efficacy to treat psoriasis and are the first line of drugs to treat psoriasis in some countries (Yost and Gudjonsson, 2009). Downstream effects of TNF-α are mainly mediated by i) NF-κB activation leading to increase in inflammatory response via IL8 and CCL20; ii) mitogen-activated protein kinase (MAPK) and c-JUN promoting cellular proliferation and differentiation; and iii) death signalling regulating cell growth survival and apoptosis (Gaur and Aggarwal, 2003, Locksley et al., 2001).

IL-23 has critical role in psoriasis initiation by regulating T_H17 differentiation and maturation (*Figure 4*) (McGeachy et al., 2009, Tonel et al., 2010). Myeloid DCs and probably keratinocytes are rich source of IL-23 in psoriasis (Di Meglio and Nestle, 2010, Li et al., 2018, Piskin et al., 2006, Ramnath et al., 2015, Tonel et al., 2010). The essential role of IL-23 in psoriasis is further supported by mouse models where intradermal injection of IL-23 or overexpression of IL-12/23p40 in murine keratinocytes resulted in the development of psoriasis-like skin inflammation (Chan et al., 2006, Di Meglio and Nestle, 2010, Lowes et al., 2014). In addition, biological treatment targeting the common p40 subunit of IL-23 and IL-12 (ustekinumab) or IL-23-specific p19 subunit (tildrakizumab, guselkumab and risankizumab)

have shown great treatment responses in treating psoriasis (Girolomoni et al., 2017, Savage et al., 2015).

IL-17A is central in psoriasis pathogenesis as biologics specifically targeting IL-17A (secukinumab: neutralizing IL-17A) have shown remarkable efficacies and have been approved to treat psoriasis (Hueber et al., 2010, Martin et al., 2013). The essential role of IL-17 in psoriasis pathogenesis is further evidenced by higher gene expression and protein levels of IL-17A, F and C in psoriatic lesions compared with non-lesional skin (Martin et al., 2013). IL-17A act on a variety of cells including keratinocytes, endothelial cells, fibroblasts, and monocytes (Harper et al., 2009, Martin et al., 2013, Yao et al., 1997). Together with IL-17F, IL-17A induces keratinocytes to produce psoriasis associated factors like cytokines, β-defensins, antimicrobial peptides (S100s protein family) and chemo-attractants such as IL-8, CCL20, and CCL2 to attract neutrophils, T_H17 cells, macrophages and monocytes (*Figure 4*) (Guttman-Yassky et al., 2008, Harper et al., 2009, Martin et al., 2013, Nograles et al., 2008). Furthermore, IL-17A synergises with TNF-α responses and activates NF-κB signalling pathway (Martin et al., 2013). Act-1 is directly downstream of IL17R which binds to the intracellular arm of the IL17R and can activate two different signalling pathways (Martin et al., 2013). Act-1 and TRAF interaction activates NF-κB pathway to induce inflammatory mediators such as IL-8, TNF and CCL20 (Schwandner et al., 2000). Act-1 can also directly bind to TRAF5 in a TRAF6-indendpendent manner thus, stabilizing the inflammatory mRNAs (Hartupee et al., 2007).

IL-17C is another member of IL-17 cytokine family produced by keratinocytes in psoriasis and has been shown to act on keratinocytes to stimulate β-defensin 2 and granulocyte colony stimulating factor (Martin et al., 2013, Ramirez-Carrozzi et al., 2011). Furthermore, keratinocyte-specific overexpression of IL-17C promoted psoriasis-like skin inflammation in mice model (Johnston et al., 2013).

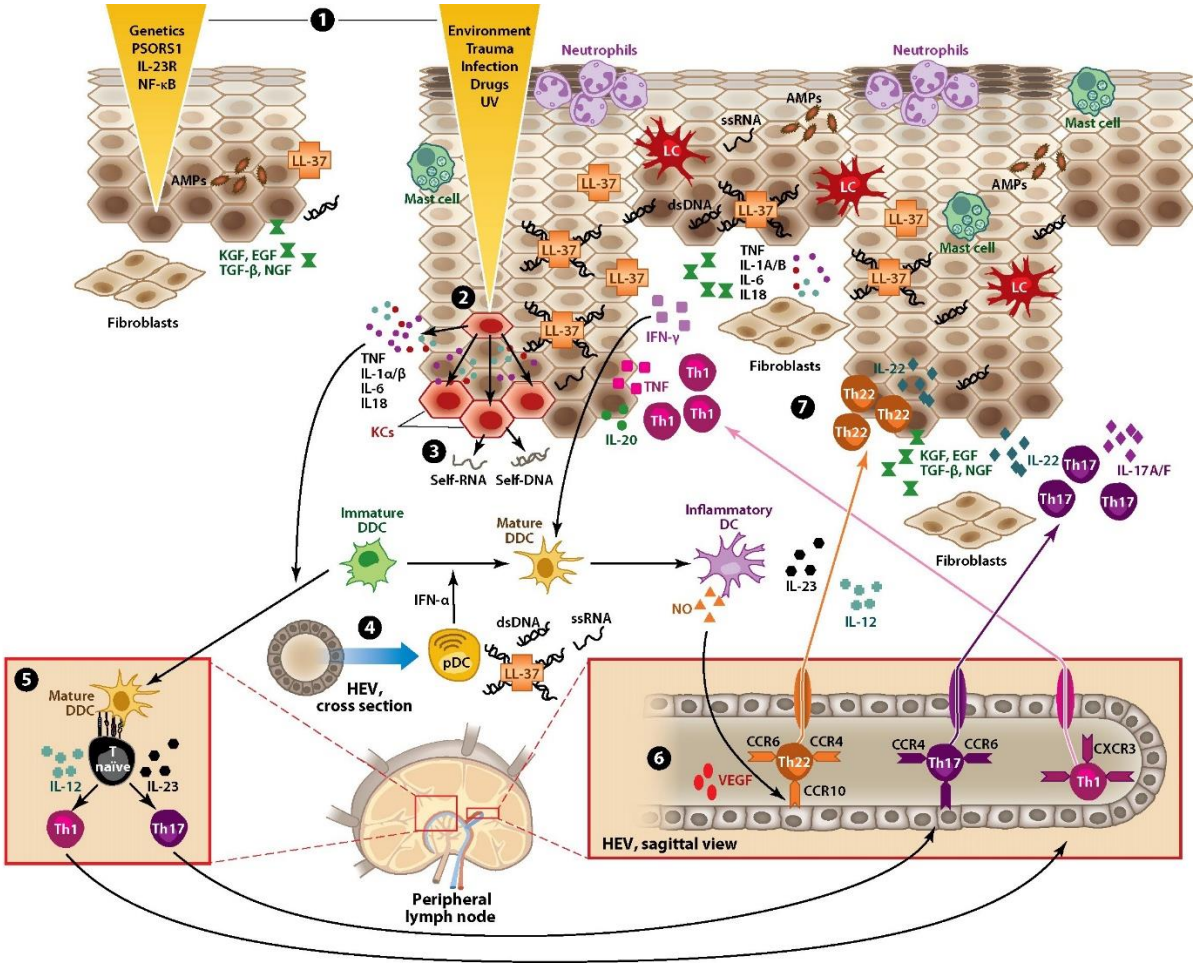


Figure 4: Model of psoriasis pathogenesis. Reproduced with permission from Perera et al., 2012. Copyright Annual Reviews.

IL-22 is produced by T_H17, T_H22, NKT and γδT cells (Zenewicz and Flavell, 2011). IL-22 belongs to the IL10 family of cytokines and binds to chains of IL10R and IL22RA1, expressed majorly on non-immune cells such as keratinocytes (Sabat et al., 2014, Zenewicz and Flavell, 2011). IL-22 is a pro-inflammatory cytokine which acts via JAK1/3/STAT3 axis and regulates expression of AMPs and MMPs (Sestito et al., 2011, Wolk et al., 2006). Histologically, IL-22 induces acanthosis in psoriasis by promoting proliferation and blocking differentiation of keratinocytes (*Figure 4*) (Boniface et al., 2005). Therapeutically, targeting JAK/STAT axis in psoriasis have been shown to be efficacious (Welsch et al., 2017).

1.2.3 Disease models of psoriasis

With the exception of a few cases in primates, psoriasis is a human specific disease (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b). Over the years several murine models have been implicated in psoriasis research with some features resembling to the human disease (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b). They can be divided in three major types: genetic model, inducible model and xenograft model (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b). In genetic models of psoriasis, researchers have targeted a specific gene(s) to observe their functional role in psoriasis pathogenesis. For example, JUN/JUNB deletion in keratinocytes led to psoriasis-like skin lesions as well as joint symptoms (psoriatic arthritis), IκB-kinase-β deletion in keratinocytes, STAT3 and TGF-β constitutive activation in keratinocytes led to spontaneous development of psoriasis-like skin inflammation (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b, Pasparakis et al., 2002, Sano et al., 2005, Zenz et al., 2005). Recently a study has shown that the keratinocyte specific overexpression of IL-17C in mouse promoted psoriasis like inflammation (Johnston et al., 2013).

A widely used inducible psoriasis model is the imiquimod-induced (TLR7/8 agonist) mouse model of psoriasis where imiquimod is applied on the ear and shaved back skin of wild type mice to generate psoriasis-like inflammation (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b, van der Fits et al., 2009). This is an acute model which recapitulates almost all the psoriasis features most importantly erythema, scaling and skin thickness together with activation of IL-23/IL-17 axis (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b, van der Fits et al., 2009). In addition, IL-23 intradermal injection in mice has also been shown to induce psoriasis-like skin inflammation (Chan et al., 2006).

In the xenograft model of psoriasis, non-lesional human skin is grafted on the back of AGR mice (lacking B and T cells and IFN-γ receptor) (Boyman et al., 2004, Gudjonsson et al., 2007,

Lowes et al., 2014, Nestle et al., 2009b, Nestle and Nickoloff, 2005). The non-lesional skin is converted to lesional skin over the weeks presenting the unique features of psoriasis like acanthosis, loss of granular layer, dermal and epidermal T cell infiltration (Boyman et al., 2004, Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b). However, the difficulty of getting large human skin for the grafting is a limiting factor of this model (Gudjonsson et al., 2007, Lowes et al., 2014, Nestle et al., 2009b).

1.3 MicroRNAs

MicroRNAs (miRNA) are among the shortest (~ 22 nucleotides in length) functional class of RNA in eukaryotes and are vital in post-transcriptional regulation of gene expression (Ameres and Zamore, 2013, Bartel, 2004, 2009). In 1993, the first ever miRNA- *lin-4* was discovered in *C. elegans* where a gene (*lin-4*) was found to encode for a small RNA rather than protein (Lee et al., 1993). Lin-4 RNA was found to regulate LIN-14 proteins through antisense complementarity at 3' untranslated region (3' UTR) of LIN-14 (Lee et al., 1993). For many years these tiny regulators were under appreciated as their existence were limited to nematodes. However, discovery of let-7 RNA in *C. elegans* (Reinhart et al., 2000, Slack et al., 2000) and homologs of let-7 in human and fly genomes (Pasquinelli et al., 2000) pointed towards an exciting class of non-coding RNAs.

To date 2588 mature human miRNAs are registered in miRbase21 (*http://www.mirbase.org/*). MiRNAs plays crucial role in regulating gene expression as evidenced by the maintained sequence complementarity among miRNAs and more than 60% of human mRNAs during the course of selection pressure through evolution (Ameres and Zamore, 2013, Friedman et al., 2009, Ha and Kim, 2014, Jonas and Izaurralde, 2015). In addition, Dicer1 knockout in mice led to lethality as Dicer1-null embryos died around 7.5 days of gestation (Bernstein et al., 2003). Similarly, antisense-mediated depletion of 46 miRNAs in early drosophila embryo led to impaired development (Leaman et al., 2005).

In humans, the majority of miRNAs are encoded in introns of non-coding or coding transcripts. Often miRNAs loci are present in close proximity and organised as a poly-cistronic transcriptional clusters which usually transcribed together (Ha and Kim, 2014). Often miRNAs which are present in the intron of protein-coding genes share the same promoter as the host genes (Monteys et al., 2010, Ozsolak et al., 2008). However, some miRNAs have multiple transcription start sites and can have their own promoter regions (Monteys et al., 2010, Ozsolak et al., 2008). Biogenesis of miRNAs is tightly regulated process which is maintained by

transcription factors (p53, MYC, ZEB1/2 and MYOD1), DNA methylation and histone modification (Davis-Dusenbery and Hata, 2010, Krol et al., 2010).

1.3.1 The biogenesis of miRNAs

In eukaryotes, miRNA biogenesis is regulated transcriptionally as well as post-transcriptionally and dysfunction in biogenesis is often related to development of disease conditions (Ameres and Zamore, 2013, Ha and Kim, 2014, Jonas and Izaurralde, 2015). MiRNAs are transcribed as long primary-miRNAs (pri-miRNAs) containing single or multiple miRNAs by RNA polymerase II (*Figure 5*) (Ameres and Zamore, 2013, Ha and Kim, 2014, Jonas and Izaurralde, 2015, Lee et al., 2004). A stem loop structure of ~ 60 nucleotide pre-miRNA is cleaved from the pri-miRNA by an RNase III enzyme Drosha in the nucleus (*Figure 5*) (Ameres and Zamore, 2013, Denli et al., 2004, Gregory et al., 2004). Drosha is a part of a bigger nuclear complex called the microprocessor, containing Drosha and double stranded (ds) RNA binding protein named DGCR8 (*Figure 5*) (Ameres and Zamore, 2013, Denli et al., 2004, Gregory et al., 2004). The excised pre-miRNAs are then transported to cytoplasm by nuclear transport receptor exportin 5 which recognises the end and the stem of the precursor-miRNA (*Figure 5*) (pre-miRNAs) (Ameres and Zamore, 2013, Okada et al., 2009, Yi et al., 2003). Once in cytoplasm, a ds-miRNA-miRNA duplex is diced from the pre-miRNAs by another RNase III enzyme called Dicer (*Figure 5*) (Ameres and Zamore, 2013, Zhang et al., 2002, Zhang et al., 2004). Dicer recognises and cuts at the certain distance from stem, liberating the loop from the pre-miRNAs (Ameres and Zamore, 2013, Zhang et al., 2002, Zhang et al., 2004). Both 5' and 3' strand of the miRNA-miRNA duplex can form a mature single strand of either 5p or 3p miRNA (Ha and Kim, 2014). After the processing of miRNAs either 5' or 3' miRNAs can be loaded to RISC complex and guide AGO proteins to the target mRNA to repress their expression via seed match sequence binding (*Figure 5*) (Ameres and Zamore, 2013). Binding of miRNAs to

mRNAs can lead to either translational repression or mRNA degradation (Ameres and Zamore, 2013).

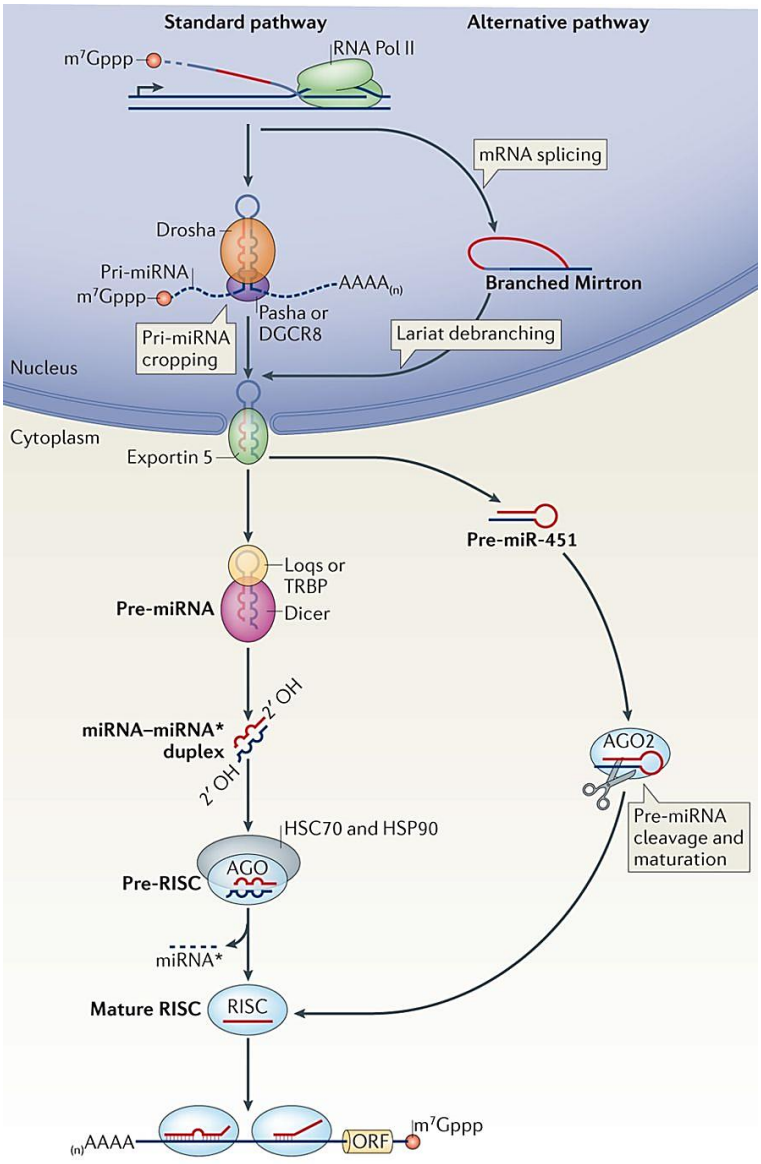


Figure 5: Schematic of miRNA biogenesis. Reproduced with permission from Ameres et al., 2013. Copyright Springer Nature.

1.3.2 Mode of action of miRNAs

MiRNAs exert their function by either directly degrading the complementary target mRNAs (Bagga et al., 2005, Giraldez et al., 2006, Jing et al., 2005, Lim et al., 2005) or blocking their translation (*Figure 6*) (Ameres and Zamore, 2013, Ha and Kim, 2014, Hausser and Zavolan, 2014, Jonas and Izaurralde, 2015). Each miRNA can regulate hundreds of genes, often these

regulated targets are within the same pathway (Bartel, 2009). MiRNAs are an integral part of RNA induced silencing complex (RISC), where they associate with Argonaute (AGO) family proteins- the catalytic unit of the RISC (*Figure 6*) (Ameres and Zamore, 2013, Ha and Kim, 2014, Jonas and Izaurralde, 2015). Regulation of mRNAs via miRNAs is mostly dependent on seed sequence of miRNAs. Seed sequence is a 6-8 nucleotide long sequence present on 5' end of the miRNAs (usually between positions 2 to 8) through which miRNAs binds to the seed matches, often present at 3' UTR of mRNAs (Ameres and Zamore, 2013, Forman and Coller, 2010, Grimson et al., 2007, Ha and Kim, 2014, Jonas and Izaurralde, 2015).

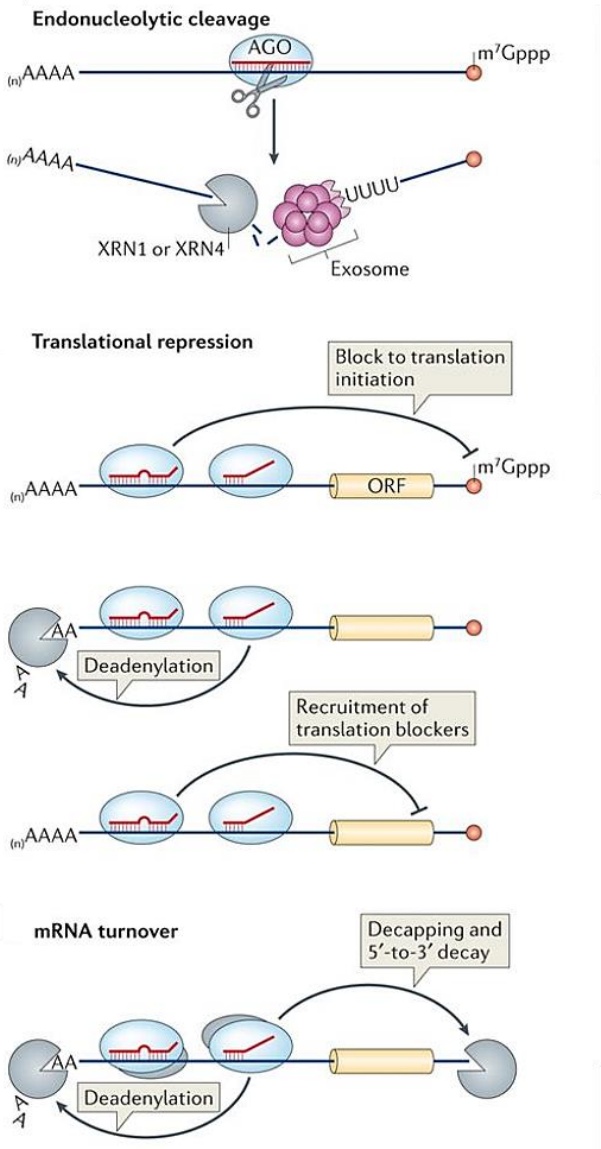


Figure 6: Mode of action of miRNAs. Reproduced and modified with permission from Ameres et al., 2013. Copyright Springer Nature.

The effects of miRNAs on the regulation of a target mRNAs is relatively mild, however often their gene targets regulate the same cellular pathways, thus even a small regulation of target mRNAs leads to a vast impact on the biological processes (Ameres and Zamore, 2013). MiRNAs with the same seed sequence belong to the same family (with overlapping target genes), while miRNAs with same origin and primary transcript belong to the same cluster (Ameres and Zamore, 2013, Ha and Kim, 2014, Jonas and Izaurralde, 2015).

MiRNA-mediated degradation of target mRNAs is directed by enzymes involved in 5'-3' mRNA decay pathway, where final degradation is facilitated by exoribonuclease 1(XRN1) (*Figure 6*) (Ameres and Zamore, 2013, Ha and Kim, 2014, Jonas and Izaurralde, 2015). Translational repression is an early event after miRNAs expression and according to evolving consensus, it supresses cap dependent translation at initiation (Jonas and Izaurralde, 2015).

1.3.3 MiRNAs in diseases

Considering the diverse expression pattern and tight regulation of miRNAs targeting a significant number of mRNA targets, it is not surprising that miRNAs have been implicated in several diseases such as cancer, cardiovascular disease, immunological disease and many other diseases (Lovendorf and Skov, 2015, Mehta and Baltimore, 2016, Mendell and Olson, 2012, Rupaimoole and Slack, 2017). First implication of miRNAs in diseases regulation originated from cancer studies. Cell-specific miRNA expression patterns are useful for tumour prognosis and classification (Calin and Croce, 2006). Tumour-suppressive miRNAs such as let-7, miR-34a and miR-145 and miR-200 family and oncomiRs such as miR-10b, miR-155 and miR-222 have been implicated in several different cancer types (Rupaimoole and Slack, 2017).

MiRNAs have also been also utilized as minimally-invasive biomarkers as they are present and are rather stable in body fluids such as blood, serum, plasma, saliva and urine (Hydbring and Badalian-Very, 2013). The extracellular miRNAs are either encapsulated in microvesicles or

bound to AGO2 protein (Turchinovich et al., 2013). The microvesicles incorporated miRNAs are thought to be a part of cell to cell communication, while AGO2 bound miRNAs are majorly the result of cell death (Turchinovich et al., 2013). In both the cases miRNAs could serve as biomarkers by reflecting a certain stage or a cellular signals.

MiRNAs have a unique expression signature in almost all the human diseases, leading to an opportunity to overexpress (with miRNA mimics) or inhibit (with miRNA inhibitors) a particular miRNA to counteract its effect in the disease scenario (Lovendorf and Skov, 2015, Mendell and Olson, 2012, Rupaimoole and Slack, 2017). Therapeutically, miRNAs has been targeted to treat different diseases, for example miR-122 for Hepatitis C infection, miR-34 for liver cancer, miR-208 for cardio-metabolic disease and miR-103/105 for insulin resistance (Elmen et al., 2008, Li and Rana, 2014, Montgomery et al., 2011, Rupaimoole and Slack, 2017).

1.3.4 MiRNAs in skin and psoriasis

Skin-specific knockout of Dicer, a key component of miRNAs biogenesis in mice resulted in a halt in production of miRNAs leading to aberrant morphogenesis of stratified epithelial tissues and neonatal conditional knockout mice did not survive past post-natal day 4-6, indicating a crucial role of miRNAs in skin (Yi and Fuchs, 2010, Yi et al., 2006). Similarly, DGCR8 skin-specific knockout mice developed rough and dehydrated skin and mice died at neonatal stages (Yi and Fuchs, 2010, Yi et al., 2009).

In 2007, our group demonstrated for the first time that miRNAs are differentially expressed in psoriatic skin (Sonkoly et al., 2007) as compared to healthy skin, but also as compared to another inflammatory skin disease, atopic dermatitis. Since then, several studies investigating global miRNA expression by microarray or RNA sequencing have shown altered miRNA levels in psoriatic plaques (Joyce et al., 2011, Lovendorf et al., 2015, Zibert et al., 2010). One of the miRNAs we identified to be up-regulated in psoriasis, miR-203, showed skin-specific

expression profile and it became the most studied miRNA in skin (Lena et al., 2008, Sonkoly et al., 2008, Sonkoly et al., 2007, Yi et al., 2008). MiR-203 plays a pivotal role in epidermal cell differentiation, stratification and skin cancer (Lohcharoenkal et al., 2016, Lovendorf and Skov, 2015, Sonkoly et al., 2012, Sonkoly et al., 2007, Yi et al., 2008). MiR-203 targets gene suppressor of cytokine signalling SOCS-3 and involved in keratinocytes differentiation and immune response (Sonkoly et al., 2007).

We and others have shown that deregulated miRNAs modulate cellular functions altered in psoriasis, such as epidermal differentiation (miR-21, miR-125b, miR-203, miR-99a) (Lerman et al., 2011, Sonkoly et al., 2007, Xu et al., 2011), T cell apoptosis (miR-21) (Meisgen et al., 2012), and keratinocyte-immune cell cross talk (miR-31) (Xu et al., 2013). Therapeutically targeting miR-21 has shown potential to treat psoriasis in psoriasis xenograft model and psoriasis-like mouse models (Guinea-Viniegra et al., 2014). MiR-146a is also upregulated in psoriasis (Sonkoly et al., 2007) and negatively regulates TLR2-induced inflammatory responses in keratinocytes (Meisgen et al., 2014). Altogether these findings indicate an important role of miRNAs in psoriasis pathogenesis, progression and maintenance.

2 AIMS

In this thesis, we aimed to decipher the regulatory networks of cytokines and miRNAs in psoriasis pathogenesis, progression and maintenance.

The objectives of this research were

- to understand role of miR-146a in psoriasis *(Paper I)*,
- to identify the miRNA landscape of keratinocytes in psoriasis *(Paper II)*,
- to explore the role of miR-149 in keratinocytes in psoriasis *(Paper III)*,
- to determine the effect of the JAK inhibitor tofacitinib on keratinocytes *(Paper IV)*.

3 MATERIAL AND METHODS

Patients and healthy donors

Four mm skin punch biopsies were collected from lesional and non-lesional skin of patients with chronic plaque psoriasis or healthy donors of Caucasian origin. Psoriasis patients went through a drug washout period of 4 weeks for systemic therapy and 2 weeks for UV treatment before sample collection. For genetic analysis, DNA was isolated from 1546 psoriasis patients and ethnically matched 1526 control subjects (Stockholm Psoriasis Cohort). All the donors provided informed consent for the study. The study was approved by the Stockholm Regional Ethics Committee and all the procedure involving human samples were performed according to the Declaration of Helsinki's principles.

Genetic analysis

To detect genetic association of single nucleotide polymorphism in miR-146a precursor and psoriasis, the rs2910164 SNP was genotyped in 1546 psoriasis patients and 1526 control subjects. The genotype was detected using QuantStudio 7 Flex and allele-specific TagMan MGB probes (Thermo Fisher Scientific, Stockholm Sweden). Allelic association of rs2910164 was performed in PLINK v1.07 by using logistic regression with sex as the covariate. Hardy-Weinberg equilibrium was evaluated using χ^2 for each SNP to establish the genetic association.

$CD45^{neg}$ cell isolation

Skin biopsies were incubated with dispase (5 U/ml) (Thermo Fisher Scientific, Stockholm, Sweden) for 14-16 hours at 4˚ C and epidermis was separated from dermis using forceps. To obtain single cell suspension, epidermal sheets were further sliced and incubated with trypsin-

EDTA (Thermo Fisher Scientific, Stockholm, Sweden) for 15 mins at 37˚ C. To tag the $CD45^{pos}$ cells, epidermal single cells were incubated with $CD45^{pos}$ microbeads (Miltenyi Biotec, Stockholm, Sweden) for 15 mins at 4˚ C. $CD45^{neg}$ cells (majorly keratinocytes) were negatively sorted using MACS MS magnetic columns (Miltenyi Biotec, Stockholm, Sweden).

Mice

Female BL6/miR-$146^{-/-}$ and wild-type (WT) C57BL/6J, 6-8 weeks old mice were obtained from Jackson laboratory (Bar Harbor, Maine, USA) and Charles River (Wilmington, Mass, USA) respectively. To mimic the initiation phase of psoriasis-like skin inflammation in mice, 5% imquimod (Aldara cream; MEDA, Stockholm, Sweden) (31.25 mg) was applied on ears for 3 consecutive days and mice were sacrificed on day 4. To study the resolution phase of psoriasis-like skin inflammation in mice, imiquimod (62.5 mg) was applied to shaved dorsal mice skin for 6 consecutive days and followed for 10 days or sacrificed at day 7. Control animals were treated with vehicle cream. Symptoms of psoriasis-like skin inflammation such as erythema, scaling, and skin thickness were scored (0-none; 1-mild; 2-moderate; 3-severe and 4- very severe) by three researchers independently. To overexpress miR-146a in skin, complex of miR-146a mimics or scramble controls (Thermo Fisher Scientific, Stockholm Sweden) with transfecting agent (In Vivo RNA-LANCEr II) (Bioo Scientific, Austin, Texas, USA) were injected intra-dermally in shaved mice dorsal skin at day 1, 2 and 4 in WT mice. Imiqimod (62.5 mg) was applied on back skin at day 2, 3 and 4 and mice were sacrificed at day 5. The animal study was approved by Local Ethics Committee of Stockholm, Sweden (Swedish Board of Agriculture).

Cell culture, treatments, and transfections

Human primary keratinocytes were obtained from Thermo Fisher Scientific (Stockholm, Sweden) and cultured in Epilife medium (Thermo Fisher Scientific, Stockholm Sweden) with 1% human keratinocyte growth supplement (HKGS) (Thermo Fisher Scientific, Stockholm, Sweden) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Stockholm, Sweden) at 37˚ C with 5% CO_2. To avoid any basal induction of cytokine production by hydrocortisone (present in HKGS) keratinocytes were incubated with EpiLife medium without any supplements during the experiments. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of healthy donors, by using Ficoll (GE Healthcare, Stockholm, Sweden) density separation.

For cytokine stimulation experiments, keratinocytes were treated with IL-17A (5, 10, 20, 40, 80, 100 or 200 ng/ml), IFN-γ (20 ng/ml), IL-1β (10 ng/ml), IL-22 (20 ng/ml), TNF-α (50 ng/ml), IL-36α (10 ng/ml) and IL-17A+IL-22+TNF-α (100 ng/ml, 10 ng/ml and 25 ng/ml) for the specified time points. All the cytokines were purchased from R&D Systems (Minneapolis, Minnesota, USA). To block JAK/STAT pathway, human primary keratinocytes were pre-treated either with JAK1/3 inhibitor- tofacitinib (0.6 μM) (Phizer, Stockholm, Sweden or Selleckchem, Houston, Texas, USA) or JAK1/2 inhibitor ruxolitinib (0.3 μM) (Selleckchem, Houston, Texas, USA) for 1 hour, followed by treatment of IL-22 or IFN-γ for 1 or 24 hours. Dimethyl sulphoxide (DMSO) (Merck, Stockholm Sweden), was used as a vehicle control for the inhibitors.

To judge the functional role of miR-146a or miR-149, third passage, 50-70% confluent human primary keratinocytes were transfected with miR-146a/miR-149 mimics (1 nM) (Thermo Fisher Scientific, Stockholm, Sweden) or miR-146a/miR-149 inhibitors (50 nM) (Qiagen, Stockholm, Sweden) or corresponding negative controls using Lipofectamine 2000 (Thermo Fisher Scientific, Stockholm, Sweden) for specified time points.

Three-dimensional (3D) epidermal equivalents were procured from MatTek (Ashland, MA, USA) and maintained at the air-liquid interphase as recommended by the manufactures. IFN-γ (20ng/ml) (R&D Systems, Minneapolis, Minnesota, USA) was added to the 3D epidermal equivalent for 72 hours. 3D-epidermal equivalents were either FFPE sectioned and stained with hematoxilin/eosin (Histolab, Stockholm, Sweden) or snap frozen for RNA isolation.

RNA extraction

From cell culture experiments, total RNA was extracted using Trizol (Thermo Fisher Scientific, Stockholm, Sweden) and chloroform (Merck, Stockholm Sweden) (phenol-chloroform). Total RNA was extracted from animal tissues using tissue-lyser (Qiagen, Stockholm, Sweden) and miRNeasy mini kit (Qiagen, Stockholm, Sweden). miRNeasy mini kit (Qiagen, Stockholm, Sweden) was also used to isolate total RNA from $CD45^{neg}$ cells or PBMCs. RNA concentration was measured using Nanodrop 2000 or Nanodrop one (Thermo Fisher Scientific, Stockholm, Sweden). For small RNA sequencing or microarray analysis RNA quality was detected using Agilent 2100 Bioanalyzer chip (Agilent, Stockholm, Sweden). RNA samples with RNA Integrity Number (RIN) more than 9 were used for small RNA sequencing or microarray.

Small RNA sequencing

Next generation sequencing for small RNAs was performed at BGI (Beijing Genomics Institute, Beijing, China) on RNA isolated from keratinocytes of healthy (n = 9), psoriasis lesional and non-lesional donors (n = 9). Adopters and low expressed tags were trimmed from the data and clean reads were aligned to miRBase to detect the known miRNAs. Clean reads for putative novel miRNAs of 22-25 nucleotides were aligned to human genome and secondary structure (precursor) was predicted using MIREAP algorithm. Differential expression of

miRNAs were analysed using Edge-R in Bioconductor (False Discovery Rate < 10%, Fold Change > 1.4). Only the miRNAs expressed as 1 transcript per million in at least half of the samples of at least one of the groups were considered to be robustly expressed and considered for the differential expression analysis.

Transcriptomic profiling

For transcriptomic analysis, RNA samples were hybridized on Affymetrix Gene Titan ST2.1 (Stockholm, Sweden) array plate and scanned using Affymetrix Gene Titan microarray scanner (Stockholm, Sweden). Data was normalized using Affymetrix standard protocol. Adjusted P-value < 0.05 or P-value < 0.05, (fold change > 1.4 or > 1.2) were calculated using SAM (significance analysis for microarray), using MEV (Multiple Experiment Viewer) (TM4) (Howe et al., 2011). Heatmaps were plotted using Morpheus (Broad Institute, https://software.broadinstitute.org/morpheus) or MEV (TM4).

Gene enrichment and gene network analysis

Enrichment analysis was performed using Gene Set Enrichment Analysis (GSEA) software (Broad Institute) (Mootha et al., 2003, Subramanian et al., 2005). KEGG pathway enrichment or gene ontology was performed using EnrichR (Ma'ayan Laboratory) (Chen et al., 2013, Kuleshov et al., 2016). Gene networks were generated using gene ontology clustering (KEGG) in Cytoscape using the ClueGo plugin (Bindea et al., 2009).

qRT-PCR

Total RNA was reverse transcribed either using miRNAs specific primers (miR-146a/miR-149/miR-941/miR-1307-3p/RNU48) and MicroRNA Reverse Transcription Kit (Thermo

Fisher Scientific, Stockholm, Sweden) or using oligo dT and random hexamer (RevertAid First Strand cDNA Synthesis Kit) for mRNAs (Thermo Fisher Scientific, Stockholm, Sweden). Expression of miR-146a, miR-149, miR-941 and miR-1307-3p was analysed with TaqMan probes using QuantStudio Flex 7 (Thermo Fisher Scientific, Stockholm, Sweden). Expression of miRNAs was normalised to RNU48 and relative expression was calculated using ΔΔCt calculation. Expression of JAK 1, JAK2, JAK3, Tyk2 (Thermo Fischer Scientific, Stockholm, Sweden), IL-8, CCL20, S100A7, S100A8, S100A9, EGR1, IL-6, CXCL9, CXCL10 and CXCL11 (Integrated DNA Technologies, Coralville, Iowa, USA) was determined using TaqMan based predesigned qPCR assays and QuantStudio Flex 7. Gene expression was normalized based on housekeeping gene 18S (18S fwd: CGGCTACCACAT CCAAGGAA; rev: GCTGGAATTACCGCGGCT, probe: TGCTGGCACCAGACTTGCCC TC) using ΔΔCt calculation. Expression of mouse *Il-1β, IL-8, Cxccl1, Ccl20, Cxcr2, S100a7, S100a8, S100a9* and *Krt16* was detected by QuantStudio Flex 7 using TaqMan based predesigned qPCR assays (Integrated DNA Technologies, Coralville, Iowa, USA). Gene expression was normalized to mouse *GAPDH*.

Immunoblotting

Cell lysate was resolved on 4-20% gradient gels (Bio-Rad, Stockholm, Sweden) and transferred on to the nitrocellulose membrane (Bio-Rad, Stockholm, Sweden). The membrane was blocked with 5% milk in phosphate buffer saline (with tween 20) for 1 hour. Immunoblotting was performed for p-p65 (1:1000), p-65 (1:1000), pSTAT3 (1:1000), STAT3 (1:1000), pSTAT1 (1:1000), STAT1 (1:1000), JAK1 (1:1000), JAK2 (1:1000) and JAK3 (1:1000) (Cell Signalling Technology, Stockholm, Sweden) at 4˚ C for 12-16 hours. Next day, membranes were washed and incubated either with horseradish peroxidase-coupled (HRP) isotype-specific anti-rabbit (1:2000) or anti-mouse (1:1000) secondary antibodies, obtained from Dako (Agilent, Stockholm, Sweden) for 1-2 hours. Chemiluminescence (GE Healthcare, Stockholm,

Sweden) detection system was used to detect the blot signals. HRP-coupled β-Actin (1:20000) (Merck, Stockholm Sweden) was used as a loading control.

Histology, immunohistochemistry and immunofluorescence

Formalin-Fixed Paraffin-Embedded (FFPE) skin sections (6-8 μM thick) were used for histology and immunohistochemistry (IHC). Mouse skin section were stained with hematoxylin and eosin (H&E) (Histolab, Stockholm, Sweden) and photographed. Epidermal thickness was measured using ImageJ software on the histology images. IHC was performed for anti-mouse Gr-1 (1:200) (rat monoclonal) and Ki67 (1:200) (rabbit monoclonal) (Cell Signalling Technology, Stockholm, Sweden) using ABC and AEC peroxidase (HRP) substrate kit (Vectastain) (Vector Laboratories, Burlingame, CA, USA). Gr-1/Ki67 positive cells were counted per field of view using ImageJ. To detect nuclear translocation of p65, cells were fixed and incubated with p65 antibody (1:500) (Cell Signalling Technology, Stockholm, Sweden) and detection was performed using Alexa Fluor 564-conjugated secondary antibody (Thermo Fisher Scientific, Stockholm, Sweden). DAPI was used for nuclear staining (Thermo Fisher Scientific, Stockholm, Sweden).

In situ hybridization

In situ hybridization for miR-146a or miR-149 was performed on 6-8 μM thick FFPE skin sections. Sections were de-paraffinized and treated with proteinase K (20 μg/ml) (Thermo Fisher Scientific, Stockholm, Sweden) for 15 mins at 37˚C and incubated with miR-146a or scramble control (55˚C) or miR-149 or scramble probe (53˚C) (double DIG) (Qiagen, Stockholm, Sweden) for overnight. Sections were washed with SSC buffer (Thermo Fisher Scientific, Stockholm, Sweden) and incubated with alkaline phosphatase-conjugated sheep

anti-digoxigenin Fab fragments (1:800) (Roche, Merck, Stockholm, Sweden) for 1 h at room temperature. Antibody conjugated probe signal was developed with BM purple alkaline phophatase substrate (Roche, Merck, Stockholm, Sweden) for overnight at room temperature.

Laser-capture microdissection

10 µM thick FFPE mouse skin sections were de-paraffinized and stained with H&E (Histolab, Stockholm, Sweden). The epidermal areas were marked with digital pen followed by laser-section microdissection using Leica LMD7000 (Leica Microsystems, Stockholm, Sweden).The laser cut epidermis pieces were collected in a tube containing Proteinase K digestion buffer (Qiagen, Stockholm, Sweden) and were further processed for RNA isolation.

ELISA

Supernatant from keratinocytes cell culture upon miRNA transfections (mimic/inhibition) followed by cytokine treatments were collected and stored in -80 ˚C. ELISA for IL-8, CCL20 or IL-6 (Biolegend, San Diego, California, USA) was performed using manufacturer's instruction.

Neutrophil migration assay

Primary human neutrophils were isolated from whole blood collected from healthy donors. Erythrocytes were removed using dextran sedimentation (2:1 mixture of blood: 6% dextran / 0.9% NaCl) (Merck, Stockholm, Sweden), followed by hypotonic lysis. Purified neutrophils were suspended in EpiLife serum-free keratinocyte growth medium, and 6×10^5 cells were added to the inner chamber of a 3 µm PET membrane cell culture insert (BD Falcon,

Erembodegem, Belgium). Cell culture supernatant from keratinocytes transfected with pre-miR-146a, pre-miR-Ctrl, anti-miR-146a or anti-miR-Ctrl followed by IL-17 treatment was added to the outer chamber. After incubation for 1 to 1.5 hours at 37° C in 5% CO2, the migrated neutrophils in the outer chamber were quantified by flow cytometry and normalized to the culture medium volume by addition of CountBright counting beads (Thermo Fisher Scientific, Stockholm, Sweden).

Statistical analysis

Student's t-test, Mann-Whitney U test, two-way ANOVA were performed using Prism 6.0 (Graph Pad Software, La Jolla, USA). P-values < 0.05 were considered to be statistically significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ****$P < 0.0001$.

4 RESULTS AND DISCUSSION

4.1 THE ROLE OF MIR-146A IN PSORIASIS

Genetic variation is one of the strongest component in psoriasis pathogenesis and in recent years several psoriasis susceptibility genes have been identified using genome wide association studies. In 2007, our group performed the first ever miRNA profiling of psoriasis skin and found miR-146a to be upregulated in psoriasis (Sonkoly et al., 2007). Since then, we and other groups have shown that miR-146a regulates innate immune functions of keratinocytes and NF-κB mediated inflammatory signalling in atopic dermatitis (Meisgen et al., 2014, Rebane et al., 2014). Moreover, naturally aged miR-146a knockout mice developed chronic inflammation indicating an important role of miR-146a in inflammation (Boldin et al., 2011, Zhao et al., 2011). In this study we aimed to investigate the association of a single nucleotide polymorphism (SNP) in miR-146a with psoriasis and investigate the function of miR-146a in the disease.

4.1.1 Results

4.1.1.1 A functional polymorphism of miR-146a is associated with psoriasis

SNP rs2910164 (G to C) is located in the precursor of miR-146a which result in altered levels of miR-146a due to G:U pair to C:U mismatch in the stem region of the miR-146a precursor (Jazdzewski et al., 2008). Genotype analysis of this SNP in the Stockholm Psoriasis Cohort including DNA samples from psoriasis patients (n=1546) and healthy controls (n=1526) revealed a small but significant protective association of the CC genotype compared to GG or GC genotype (P=0.03 odds ratio=0.68 95% confidence interval 0.47-0.97) (Paper I, Table 1). Because of the strong effect of the *HLA-C*06* risk gene for psoriasis, which might hide less strong, but significant effect of other genes, next we decided to stratify our cohort based on

*HLA-C*06* status. Strikingly we observed a strong protective association of rs2910164 to psoriasis in *HLA-C*06*-negative patients (P=0.008 odds ratio=0.53 95% confidence interval 0.33-0.84) (Paper I, Table 1). Moreover, our analysis revealed a strong protective association of rs2910164-CC genotype in psoriasis which protects against the early onset of psoriasis (Paper I, Table 1). Altogether, these results indicate a protective association of rs2910164-CC genotype to psoriasis.

4.1.1.2 miR-146a knockout mice develop earlier onset of imiquimod- induced psoriasis-like skin inflammation

Next, we aimed to investigate the role of miR-146a in psoriasis *in vivo*. To this end, imiquimod (a ligand of TLR 7/8, and a known inducer of psoriasis-like skin inflammation) (van der Fits et al., 2009) was applied on the ear of 6-8 week old female BL6/miR-146$^{-/-}$ and wild-type (WT) C57BL/6J for three consecutive days (*Figure 7A*, Paper I, Figure 1A).

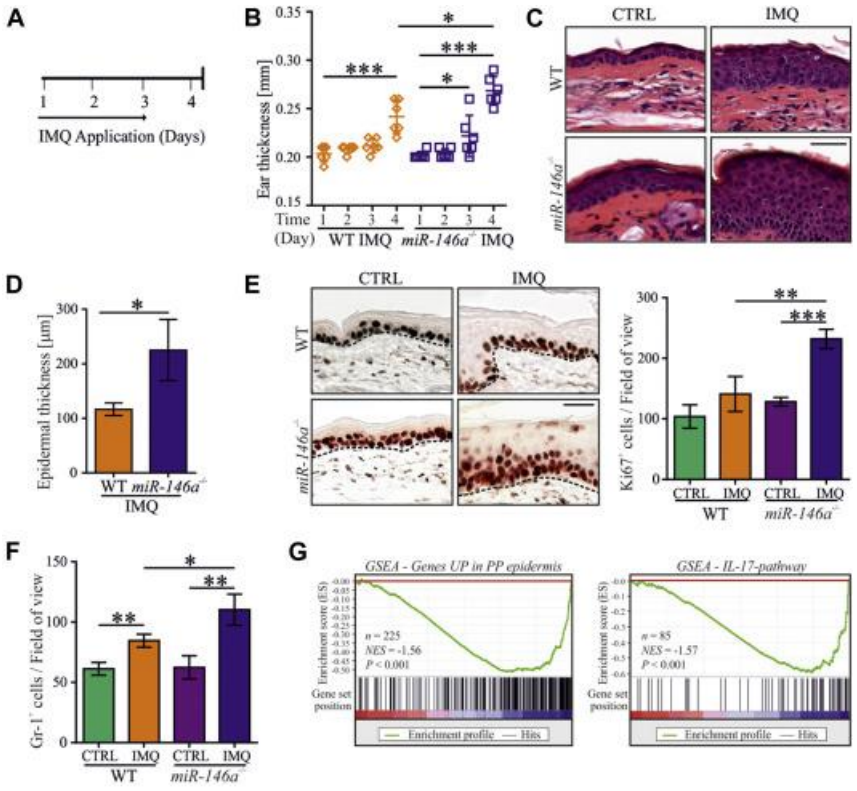


Figure 7: (A) Experimental setup. (B) Skin thickness measured over a course of 4 days of

imiquimod treatment. (C-D)Histology for mice skin and quantification of epidermal thickness. (E-F) Immunohistochemistry for Ki67 and Gr1. (G) GSEA analysis of psoriasis epidermal and IL-17A pathway gene enrichment among the deregulated genes in BL6/miR-146$^{-/-}$ mice ear upon imiquimod treatment. Reproduced with permission from Srivastava et al., 2016. Copyright Elsevier.

BL6/miR-146$^{-/-}$ mice showed an earlier onset of the disease and a more pronounced inflammation compared to the WT mice, assessed by ear thickness which is a commonly used measure of skin inflammation in this model (*Figure 7B*, Paper I, Figure 1B). Histological analysis of the ear skin revealed an increased epidermal thickness in the BL6/miR-146$^{-/-}$ mice compared to the WT mice (*Figure 7C-D*, Paper I, Figure 1C-D). Immunohistochemistry for proliferation marker Ki67 and neutrophil marker Gr1 depicted increased number of Ki67 positive and Gr1 positive cells in BL6/miR-146$^{-/-}$ mice, suggesting that keratinocytes were more proliferative upon imiquimod application in BL6/miR-146$^{-/-}$ mice and there were higher infiltration of neutrophils in these mice (*Figure 7E-F*, Paper I, Figure 1E-F). Furthermore, GSEA analysis on whole upregulated transcriptome of BL6/miR-146$^{-/-}$ mice demonstrated a significant enrichment of genes upregulated in psoriasis epidermis along with IL-17 pathway genes compared to the WT mice (*Figure 7G*, Paper I, Figure 1G).

4.1.1.3 miR-146a regulates the sensitivity of keratinocytes to IL-17A

As genes regulated by IL-17A were enriched among the deregulated genes in BL6/miR-146$^{-/-}$ mice as compared to control mice in the imiquimod-induced model, we next hypothesized that miR-146a could regulate IL-17A pathway response. To test this, we isolated primary keratinocytes from miR-146$^{-/-}$ mice or WT mice and treated them with IL-17A for 6 hours. We tested the expression of *Cxcl1*, an IL-17A response gene and a chemoattractant for neutrophils, using qRT-PCR. IL-17A induced the expression of *Cxcl1* in both WT and miR-146a-defecient keratinocytes, however the IL-17A-mediated induction of *Cxcl1* was

significantly higher in miR-146$^{-/-}$ keratinocytes compared to WT keratinocytes, suggesting that the deletion of miR-146a increases the keratinocyte sensitivity to IL-17A (Paper I, Figure 2A). In order to judge the global impact of miR-146a on IL-17 pathway next, GSEA of IL-17A regulated genes was performed using deferentially expressed genes upon overexpression of miR-146a in normal human primary keratinocytes (Meisgen et al., 2014). GSEA analysis on the whole transcriptome revealed a global impact of miR-146a overexpression on IL-17A pathway as the genes mediating IL-17A effects were significantly negatively enriched in miR-146a mimic treated keratinocytes (Paper I, Figure 2B).

Next we treated human primary keratinocytes with IL-17A in a time course fashion and tested the expression of miR-146a and human ortholog of *Cxcl1*, IL-8 using qRT-PCR. Notably, IL-17A induced IL-8 expression as early as 3 hours post-treatment with a maximal expression at 6 hours. IL-17A induced miR-146a expression 6 hours post-treatment. A sudden drop in the expression of IL-8 was observed after the miR-146a upregulation suggesting a negative regulation of IL-8 by miR-146a (Paper I, Figure 2C). To test this hypothesis in detail we treated human primary keratinocytes with different doses of IL-17A upon overexpression or inhibition of miR-146a. qRT-PCR and ELISA analysis demonstrated that the overexpression of miR-146a suppressed the IL-17A-induced expression and secretion of IL-8 (Paper I, Figure 2D). On contrary to this, inhibition of endogenous miR-146a levels in keratinocytes enhanced the IL-17A-induced expression and secretion of IL-8 (Paper I, Figure 2E). EC50 calculation for IL-17A revealed that miR-146a inhibition significantly doubled the sensitivity of keratinocytes towards the cytokine treatment in terms of IL-8 expression (Paper I, Figure E7).

Studies have shown that IL-17A effects are majorly mediated by phosphorylation and nuclear translocation of NF-κB p65 subunit. To test the effects of miR-146a on IL-17A signalling next we tested the phosphorylation status of p65 upon IL-17A induction in combination with miR-146a overexpression or inhibition. As expected, IL-17A strongly induced p65 phosphorylation in the cells treated with control oligonucleotide (Paper I, Figure 2F). IL-17A-induced

phosphorylation of p65 was decreased upon miR-146a overexpression while miR-146a inhibition further enhanced IL-17A-induced phosphorylation of p65 (Paper I, Figure 2F). In summary these results point towards an impact of miR-146a on IL-17A mediated effects in keratinocytes and suggest a potential role of miR-146a, as a negative feedback regulator of IL-17 pathway.

4.1.1.4 miR-146a knockout mice present delayed resolution of inflammation

The Imiquimod-induced mouse model of psoriasis-like inflammation is an acute model and mice spontaneously heal after discontinued application of imiquimod. IL-17A has been shown to be critical in psoriasis pathogenesis and imiquimod-induced psoriasis-like skin inflammation (van der Fits et al., 2009). As we observed a negative regulation of IL-17A pathway by miR-146a, we hypothesize that the BL6/miR-146$^{-/-}$ mice could have more severe skin inflammation and a faulty spontaneous healing. To test this, we applied imiquimod on the shaved back skin of BL6/miR-146$^{-/-}$ mice and WT mice for 6 consecutive days and followed the spontaneous healing period up to 10 days by observing features of psoriasis-like skin inflammation such as erythema, scaling and thickness (*Figure 8A*, Paper I, Figure 4A).

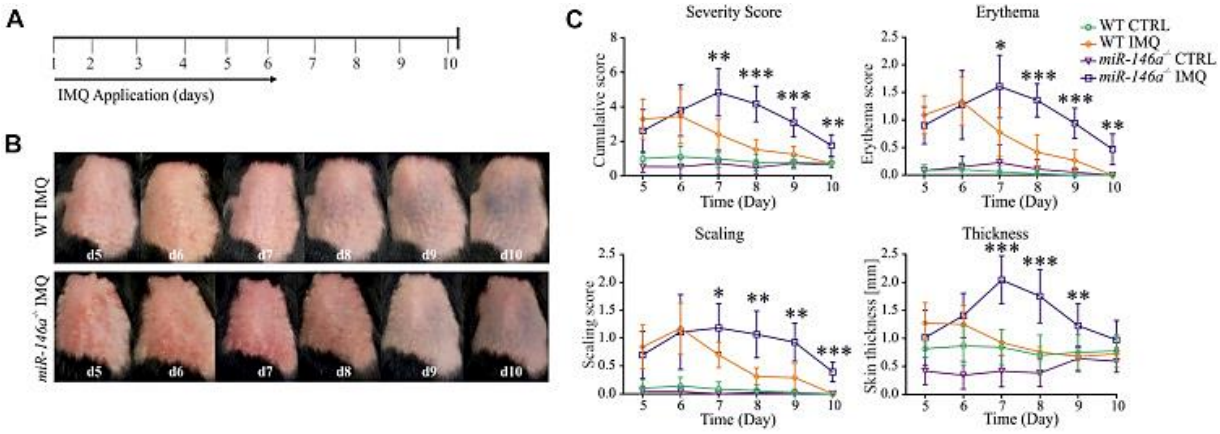


Figure 8: (A) Experimental setup. (B-C) Macroscopic image of the imiquimod treated mice along with the individual or cumulative scoring for erythema, scaling and skin thickness. Reproduced with permission from Srivastava et al., 2016. Copyright Elsevier.

Both BL6/miR-146$^{-/-}$ and WT mice developed skin inflammation, however the symptoms of psoriasis-like skin inflammation were more severe in BL6/miR-146$^{-/-}$ mice (*Figure 8B-C*, Paper I, Figure 4B-C). Scoring for erythema, scaling and skin thickness showed that the WT mice regained the normal skin at the 8th day (*Figure 8C*, Paper I, Figure 4C). While WT mice could completely resolve imiquimod-induced skin inflammation by day 10, miR-146 knockout mice still showed persistent signs of inflammation such as erythema, scaling and thickness (*Figure 8C*, Paper I, Figure 4C). In conclusion, BL6/miR-146$^{-/-}$ mice presented a more severe inflammation and incomplete spontaneous healing after discontinuing of the imiquimod treatment.

4.1.1.5 Local delivery of miR-146a mimics in mice reduces psoriasis-like skin inflammation

Our results pointed towards a protective role of miR-146a in psoriasis. Next we sought to investigate the therapeutic potential of miR-146a. To this end miR-146a mimics or scramble oligonucleotides were injected to the shaved back skin of the WT mice 1 day prior to the imiqimod application, first day and third day imiqimod application using *in vivo* transfecting agent (*Figure 9A*, Paper I, Figure 5A).

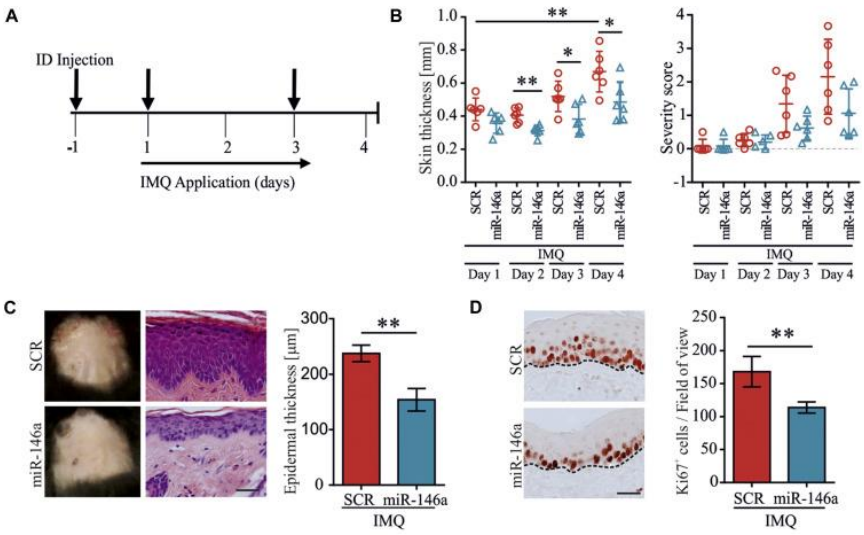


Figure 9: (A) Experimental setup. (B) Skin thickness measured over a course of 4 days of

imiquimod treatment. (C)Histology for mice skin and quantification of epidermal thickness. (D) Immunohistochemistry for Ki67. Reproduced with permission from Srivastava et al., 2016. Copyright Elsevier.

Skin thickness and severity score revealed that mice injected with miR-146a mimics were protected against imiquimod-induced psoriasiform skin inflammation compared to the mice injected with scramble control (*Figure 9B*, Paper I, Figure 5B). Histology of skin section depicted a thinner epidermis in mice injected with miR-146a mimics compared to the scramble injected mice (*Figure 9C*, Paper I, Figure 5C). In addition, immunohistochemistry demonstrated lower number of Ki67 positive keratinocytes in miR-146a mimic injected mice, suggesting a protective role of miR-146a (*Figure 9D*, Paper I, Figure 5D). In conclusion, these results suggested that local delivery of miR-146a could alleviate psoriasis-like skin inflammation.

4.1.2 Discussion

Genetic variation among psoriasis patients is critical in disease predisposition (Ray-Jones et al., 2016). To date, several psoriasis susceptibility regions have been identified and genetic variation in major histocompatibility complex (MHC) class I (*HLA-C*06)* confers the major risk for psoriasis (Ray-Jones et al., 2016). Notably, very little is known about the genetic variation among the non-coding RNA leading to psoriasis susceptibility (Pivarcsi et al., 2014). The SNP rs2910164 is located in the stem region of the miR-146a precursor and a change from G to C in this region result in higher levels of mature miR-146a (Jazdzewski et al., 2008). In line with previous studies, our data also demonstrated higher mature levels of miR-146a in psoriasis patients with rs2910164-CC genotype than the rs2910164-GG genotype. A protective association of rs2910164 to psoriasis was observed in our Swedish psoriasis patient cohort. We observed a strong protective association of rs2910164 to psoriasis upon stratification in *HLA-C*06*-negative patients and in patients with early onset of the disease. Interestingly, the protective C allele of rs2910164 is major allele in Chinese Hans population, where psoriasis

incidence rate is less than 1% (Zhang et al., 2014). In this population G allele is minor and GG/GC genotype of rs2910164 confer increased risk of psoriasis (Zhang et al., 2014). Remarkably, in Caucasian population G allele for rs2910164 is the major allele and psoriasis incidence rate is around 3-4% (Naldi, 2004), suggesting a possible protective role of miR-146a in psoriasis.

In accordance with the protective effect of rs2910164, which allows higher expression of mature miR-146a, the deletion of miR-146a in mice led to earlier onset of the imiquimod-induced psoriasiform skin inflammation. An essential hallmark of psoriasis is keratinocyte hyper proliferation (Lowes et al., 2014). Notably, deletion of miR-146a in mice led to higher number of Ki67 positive keratinocytes in epidermis of miR-146a-deficiant mice which further increased upon imiquimod induction, suggesting a role of miR-146a in regulating keratinocytes proliferation. A possible mechanism for increased proliferation could be explain by the previously identified targets of miR-146a in keratinocytes such as EGFR, FERMT1 and IL-8 which are important for the positive regulation of cell proliferation (Hermann et al., 2017, Rennekampff et al., 2000, Zhang et al., 2014). Imiquimod-induced mouse model of psoriasis is an acute model of the disease and mice spontaneously heal upon discontinuation of the imiquimod application (van der Fits et al., 2009). However, miR-146a knockout mice presented severe inflammation and delayed resolution of the skin inflammation after the discontinuation of the imiquimod application**.** As shown before (van der Fits et al., 2009), WT mice spontaneously healed by day 8 of the experiment. Our results demonstrate that the deletion of miR-146a in mice prime the skin for inflammatory triggers. Our results showing enhanced skin inflammation in miR-146a knockout mice are in line with the previous findings, showing spontaneous development of chronic inflammation in aged miR-146a knockout mice resembling the autoimmune disease, systemic lupus (Boldin et al., 2011).

Our results indicated that miR-146a negatively regulated IL-17A response pathway and inhibition of miR-146a primed the keratinocytes for inflammation. Functionally, miR-146a

regulated neutrophil chemotaxis which could be relevant to psoriasis as neutrophils are present in high number in psoriasis. MiR-146a is a strong negative regulator of NF-κB pathway and targets multiple genes in this pathway such as TRAF6 and IRAK1 (Meisgen et al., 2014). Our results indicated negative regulation of basal and IL-17A-induced p65 phosphorylation and inflammatory mediators IL-8 and CCL20 by miR-146a.

Although miR-146a is overexpressed in psoriasis (Sonkoly et al., 2007), there is still an activation of IL-17A/NF-κB axis (Martin et al., 2013). A possible explanation for this discrepancy could be explained by the multifactorial aetiology of psoriasis and the anti-inflammatory effects of miR-146a is simply not enough to completely halt the skin inflammation in psoriasis.

Therapeutically, miR-146a mimic injection in WT mice ameliorates against imiquimod-induced psoriasiform skin inflammation. In addition, application of miR-146a mimics using cell penetrating peptide PepFect6 in mice was successfully shown to deliver the miR-146a mimics to the skin leading to negative regulation of NF-κB axis (Urgard et al., 2016). The results with the miR-146a delivery with the liposome based injection or PepFect6 in mice demonstrated potential anti-inflammatory effects of miR-146a, which is encouraging and could be utilize in future to treat psoriasis alone or in combination with other available therapies.

In summary, our results identified a protective association of miR-146a SNP (rs2910164) to psoriasis. Functionally, miR-146a was found to be a potent suppressor of IL-17A-mediated skin inflammation. IL-17A induce NF-κB pathway in keratinocytes, leading to upregulation of miR-146a (*Figure 10*). MiR-146a act as a negative feedback regulator of IL-17A/NF-κB axis and act as a break on inflammation (*Figure 10*).

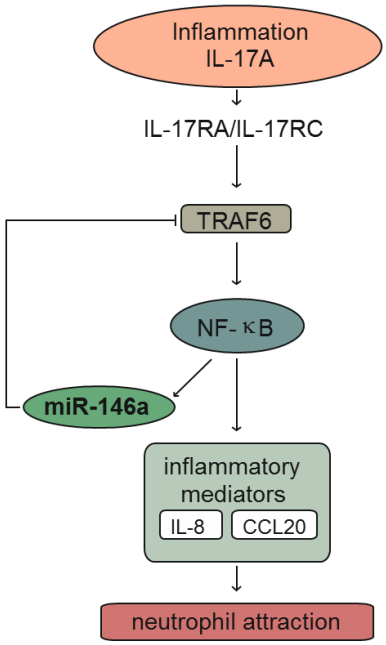


Figure 10: Schematic of miR-146a regulation and function.

4.2 MIRNA LANDSCAPE OF PSORIATIC KERATINOCYTES

Communication between keratinocytes and immune cells is an essential element regulating psoriasis pathogenesis. Despite being the key structural cells in presenting the hallmarks of psoriasis such as acanthosis, parakeratosis, and papillomatosis (Lowes et al., 2014, Perera et al., 2012), for several years, keratinocytes were thought to be mere passive bystanders in psoriasis. Recent studies demonstrated the ability of keratinocytes to produce antimicrobial peptides, cytokines and chemokines to communicate with the immune cells, thus regulating psoriasis progression and maintenance (Lowes et al., 2014, Nestle et al., 2009b, Perera et al., 2012).

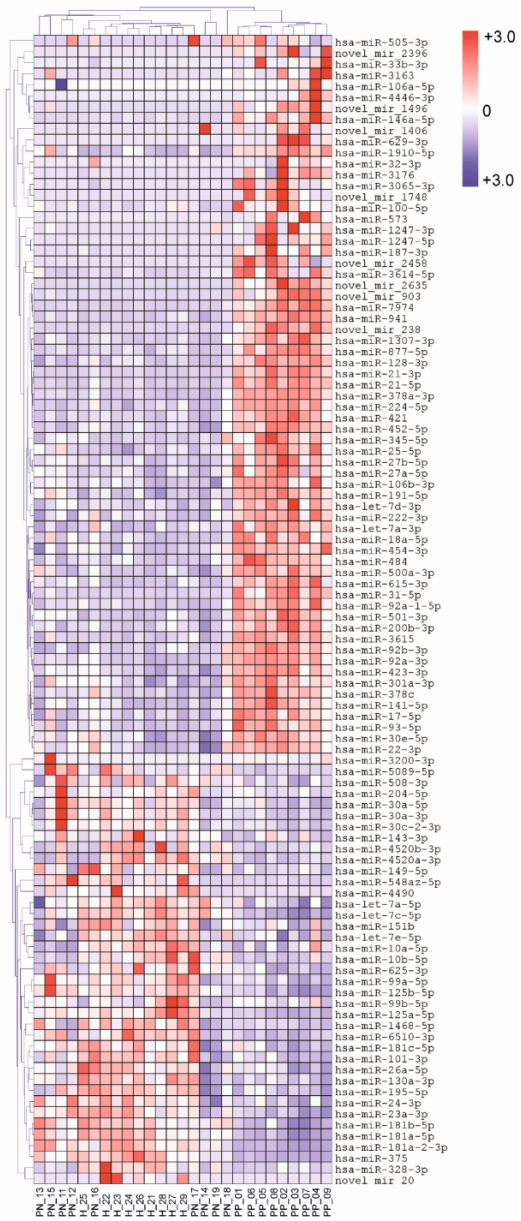
MiRNAs have been implicated in regulating keratinocyte proliferation, differentiation, and keratinocyte response to cytokines (Lerman et al., 2011, Meisgen et al., 2014, Srivastava et al., 2017, Xu et al., 2011, Xu et al., 2013). Studies dissecting the global miRNA signature of psoriasis were performed mostly by utilizing full depth skin biopsies (Joyce et al., 2011, Sonkoly et al., 2007, Zibert et al., 2010) or in one case laser-dissected psoriasis epidermis (Lovendorf et al., 2015) which contains a cellular pool of immune and structural cells from epidermis and dermis, thereby masking the cell-specific changes in miRNA landscape. In this study, we therefore aimed to identify keratinocyte-specific alterations in miRNA signature of psoriasis.

4.2.1 Results

To this end, skin biopsies were collected from psoriasis lesional as well as non-lesional skin (n=9) and healthy donors (n=9). Epidermis was separated from dermis and $CD45^{neg}$ cells (predominantly keratinocytes) were negatively sorted from epidermal sheets using magnetic MACS MS columns. Total RNA was extracted and small RNA sequencing was performed (Paper II, Supplementary Fig. S1). On an average 20 million clean reads were obtained per

sample for small RNAs and analysis of the results revealed 411 known and 30 putative novel robustly expressed miRNAs. In addition, a 3' end heterogeneity was observed in the canonical miRNAs and isomiRs with 3' end modification (3' addition or 3' deletion) were significantly enriched in psoriatic keratinocytes (Paper II, Supplementary Fig. S2). Analysis of small RNA sequencing data by EdgeR (Bioconductor) revealed differential expression of 104 miRNAs in psoriatic keratinocytes compared to healthy keratinocytes (*Figure 11a-c*, Paper II Figure 1a-c).

a



b

miRNA	FC (Lin)	P-value	FDR
novel_mir_2635	3325.482	4.589E-23	3.373E-21
novel_mir_1496	1707.640	5.501E-07	0.00001
hsa-miR-1247-3p	768.077	0.00009	0.00079
hsa-miR-1247-5p	545.741	0.00001	0.00008
novel_mir_1406	499.383	0.00186	0.01001
novel_mir_2396	360.475	0.00002	0.00026
hsa-miR-4446-3p	360.440	0.00069	0.00449
hsa-miR-3065-3p	311.774	0.00016	0.00140
hsa-miR-3163	311.661	0.00078	0.00493
hsa-miR-573	311.172	0.00078	0.00493
hsa-miR-505-3p	310.044	0.00008	0.00076
novel_mir_1748	309.433	0.00062	0.00429
hsa-miR-33b-3p	240.287	0.00036	0.00293
hsa-miR-32-3p	204.707	0.00098	0.00590
hsa-miR-629-3p	182.474	0.00139	0.00797
novel_mir_2458	144.160	0.00007	0.00071
hsa-miR-3200-3p	141.889	0.00271	0.01373
hsa-miR-106a-5p	109.109	0.00178	0.00968
novel_mir_903	65.909	0.00212	0.01114
novel_mir_238	47.646	0.00146	0.00825
hsa-miR-7974	36.272	2.438E-06	0.00004
hsa-miR-31-5p	26.058	1.845E-17	9.041E-16
hsa-miR-3176	21.073	0.01532	0.06314
hsa-miR-21-3p	17.351	3.251E-24	3.584E-22
hsa-miR-187-3p	12.226	0.01775	0.07116
hsa-miR-18a-5p	8.097	0.01968	0.07625
hsa-miR-3614-5p	7.869	0.00007	0.00071
hsa-miR-21-5p	6.146	1.708E-24	2.510E-22
hsa-miR-146a-5p	5.427	3.077E-10	9.047E-09
hsa-miR-615-3p	4.946	0.00323	0.01582

c

miRNA	FC (Lin)	P-value	FDR
hsa-miR-143-3p	0.4049	0.00164	0.00918
hsa-miR-23a-3p	0.3988	4.528E-13	1.664E-11
hsa-miR-151b	0.3959	0.01379	0.05793
hsa-miR-24-3p	0.3941	2.438E-06	0.00004
hsa-miR-508-3p	0.3742	0.00096	0.00589
hsa-miR-101-3p	0.3526	4.113E-09	1.008E-07
hsa-miR-10a-5p	0.3348	0.02262	0.08595
hsa-miR-181b-5p	0.3284	8.079E-12	2.741E-10
hsa-miR-125b-5p	0.3228	4.409E-13	1.664E-11
hsa-miR-625-3p	0.3224	0.00068	0.00449
hsa-miR-30a-5p	0.3195	0.00003	0.00030
hsa-let-7c-5p	0.3000	1.579E-13	6.961E-12
hsa-miR-195-5p	0.2898	0.00047	0.00340
hsa-miR-204-5p	0.2503	0.01677	0.06783
hsa-miR-1468-5p	0.2140	0.00003	0.00038
hsa-miR-30a-3p	0.2028	1.105E-07	2.320E-06
hsa-miR-125a-5p	0.1930	1.451E-21	9.144E-20
hsa-miR-30c-2-3p	0.1928	0.00041	0.00321
hsa-miR-181a-5p	0.1897	9.510E-24	8.387E-22
hsa-miR-328-3p	0.1637	0.00371	0.01758
hsa-miR-6510-3p	0.1476	1.690E-08	3.726E-07
hsa-miR-10b-5p	0.1338	0.00007	0.00073
hsa-miR-4520b-3p	0.1322	0.02521	0.09266
hsa-miR-4520a-3p	0.1294	0.00554	0.02546
hsa-miR-548az-5p	0.1231	0.01206	0.05265
hsa-miR-181a-2-3p	0.1156	5.779E-29	1.274E-26
hsa-miR-375	0.1002	1.405E-37	6.197E-35
hsa-miR-5089-5p	0.0654	0.00703	0.03198
novel_mir_20	0.0024	0.00037	0.00295
hsa-miR-4490	0.0007	4.980E-10	1.373E-08

Figure 11: a) miRNA signature of psoriatic keratinocytes compared to healthy keratinocytes (FC > 1.4; FDR < 0.1). (b-c) List of top 30 up and downregulated miRNAs based on fold change.

In addition, expression of 87 miRNAs were altered in psoriasis lesional keratinocytes compared to non-lesional keratinocytes (Paper II, Supplementary Fig. S3a). Interestingly, 7 miRNAs were differentially expressed in keratinocytes sorted from non-lesional psoriasis skin compared to normal keratinocytes, suggesting preclinical intrinsic changes in miRNA signature (Paper II, Supplementary Table. S3). Our analysis also identified few miRNAs whose expression could be altered in psoriatic keratinocytes due to the genetic variation in psoriasis (Paper II, Supplementary Table S7).

Strikingly, we identified several miRNAs (32 up-regulated and 18 down-regulated miRNAs in the PP vs H comparison and 36 upregulated and 18 downregulated miRNAs in the PP vs. PN comparison), which have not been identified by the previous studies using full-depth biopsies (Joyce et al., 2011, Sonkoly et al., 2007, Zibert et al., 2010) or micro-dissected epidermis (Lovendorf et al., 2015) (Paper II, Supplementary Figure 5a-d, Supplementary Table S4-S5).

Next we selected two upregulated miRNAs (miR-941 and miR-1307-3p) for validation with qRT-PCR in an extended validation cohort (H=19, Psoriasis (PP=19; PN=19). Human-specific miR-941 was found upregulated in psoriatic keratinocytes and was not identified by any earlier global miRNAs profiling studies in psoriasis. MiR-1307-3p was also found to be upregulated in psoriatic keratinocytes and was not characterized before. qRT-PCR analysis confirmed upregulation of both the miRNAs in psoriatic keratinocytes compared to normal keratinocytes (Paper II, Figure 2a-b).

4.2.2 Discussion

In the past, several studies have explored the miRNome of psoriasis by using either small RNA sequencing or microarray and reported altered expression of miRNAs in the lesional skin (Joyce et al., 2011, Lovendorf et al., 2015, Sonkoly et al., 2007, Zibert et al., 2010). Apart from identifying known signature of miRNAs in psoriasis such as miR-21, miR-31, miR-146a, miR-

125b and miR-99a (Meisgen et al., 2014, Meisgen et al., 2012, Srivastava et al., 2017, Xu et al., 2011, Xu et al., 2013) our results identified a large number of keratinocyte-specific alteration in miRNAs expression which were not identified by previous studies. A possible explanation for this could be the use of full depth skin or epidermis which contains a mix of different cell types such as keratinocytes, immune cells, melanocytes and fibroblast. The abundance of miRNAs may be different in these cell types and keratinocyte-specific changes in miRNAs expression can be compensated by increased expression in other cell types, or increased proportion of other cell types expressing the miRNA.

A small part of deregulated miRNA expression in psoriatic keratinocytes can be explained by their genomic location which coincided in the proximity of genetic variation in psoriasis. The altered expression of these miRNAs could be due the presence of lead SNP in an enhancer or silencer regions which could regulate the miRNA expression (Tsoi et al., 2017). Rest of the changes in miRNA expression may be explained either by epigenetic regulation or the inflammatory cytokine milieu of psoriasis.

In conclusion, our study dissected the miRNA landscape of keratinocytes in psoriasis, which could serve as platform for future functional studies exploring the role of miRNAs in psoriasis. Genetic variation could contribute to some of the changes in miRNA expression. In addition we also validated the expression of the human-specific miRNA-miR-941 and miR-1307-3p using qRT-PCR which could mediate keratinocyte response in psoriasis. Overall, our results may provide an insight for the future research targeting keratinocytes-specific miRNA changes for developing topical therapies.

4.3 MIR-149 REGULATES THE JAK-STAT PATHWAY IN KERATINOCYTES

We identified miR-149 as one of the downregulated miRNA in sorted keratinocytes from psoriasis lesions compared to keratinocytes isolated from healthy donors in paper II. In this study (paper III), we investigated the role of miR-149 in psoriasis pathogenesis.

4.3.1 Results

4.3.1.1 MiR-149 is downregulated in psoriasis

Our small RNA sequencing analysis identified downregulation in the expression of miR-149 in keratinocyte in psoriasis (Paper II Figure 1a, Supplementary Table S5b, and *Figure 12a*, Paper III Figure 1a). The downregulation of miR-149 was further validated by qRT-PCR in an extended cohort of sorted keratinocytes from psoriasis (L = 20, NL = 20) and healthy skin (H = 19) (*Figure 12b*, Paper III Figure 1b). The localization of miR-149 in epidermal compartment and downregulation in psoriasis lesions were further confirmed with LNA-based *in situ* hybridization (*Figure 12c*, Paper III Figure 1c).

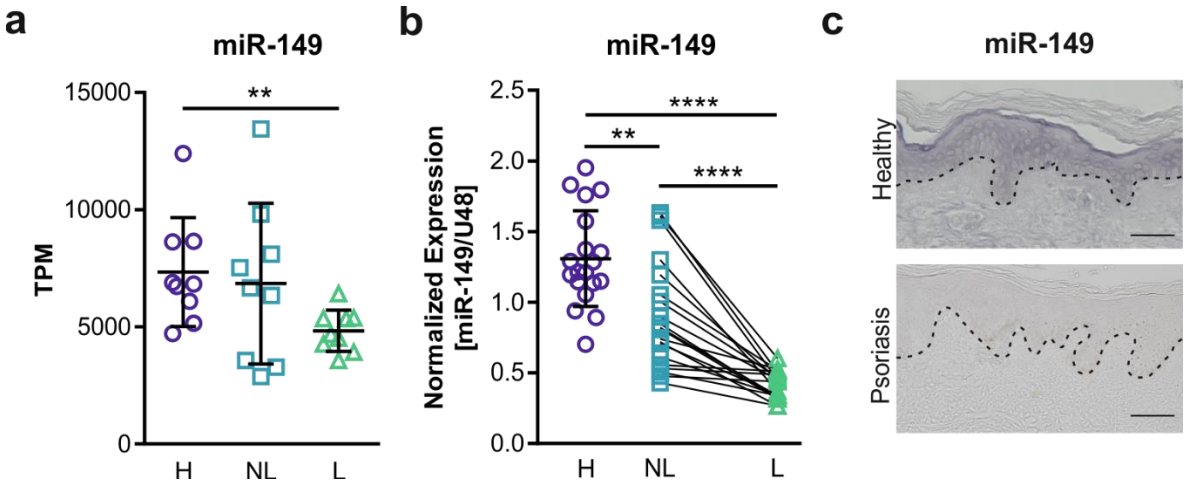


Figure 12: (a) Normalized read count of miR-149 in small RNA sequencing. (b) Expression analysis of miR-149 by qRT-PCR. (c) In situ hybridization for miR-149 in healthy and psoriasis skin.

4.3.1.2 IFN-γ suppresses miR-149 expression via the JAK/STAT-signalling pathway

Next to detect possible factors contributing to the altered expression of miR-149 in psoriasis, we treated human primary keratinocytes with psoriasis associated cytokines such as IFN-γ, IL-1β, IL-17A, IL-22, TNF-α, IL-36α or a combination of IL-17A+IL-22+TNFα. INF-γ but not the other tested cytokines regulated miR-149 expression. IFN-γ significantly downregulated miR-149 expression at 24, 48, 72 and 96 hours after treatment (Paper III Figure 2a, Supplementary Figure S1). Next, to test if the downregulation of miR-149 is transcriptional or posttranscriptional modulation by IFN-γ in keratinocytes, we detected the expression of primary transcript of miR-149 (pri-miR-149) in a time course manner. Notably, pri-miR-149 was downregulated as early as 1 hour after IFN-γ treatment, suggesting a transcriptional effect of IFN-γ on miR-149 expression (Paper III Figure 2b).

In parallel, known IFN-γ-inducible genes, CXCL9, 10, 11 and IL-6 were induced as early as 1 to 3 hours after IFN-γ treatment in monolayer keratinocytes and maximal expression of these inflammatory mediators was observed at 24 hours. Thus, miR-149 downregulation by IFN-γ at 24 hours coincided with the maximal expression of CXCL9, 10, 11 and IL-6 (Paper III Figure 2a-b) suggesting that miR-149 may regulate inflammatory pathways downstream of IFN-γ. Moreover, IFN-γ was also able to downregulate miR-149 expression in 3D epidermal equivalents at 72 hours suggesting that effects of IFN-γ on miR-149 expression is not just limited to monolayer culture of keratinocytes (Paper III Figure 3b).

Keratinocytes expresses IFN-γ receptor, which upon binding to IFN-γ activates JAK/STAT signalling via JAK1 and JAK2 leading to the phosphorylation of STAT1. Next we tested whether blocking of JAK proteins can rescue IFN-γ-suppressed miR-149 expression. To this end, human primary keratinocytes were pre-treated with ruxolitinib (JAK1 and JAK2 inhibitor) or tofacitinib (JAK1 and JAK3 inhibitor) for 1 hour, prior to IFN-γ treatment. Both ruxolitinib and tofacitinib rescued IFN-γ-suppressed miR-149 expression (Paper III Figure 4a).

Remarkably, treatment with both of these inhibitors in the absence of any other treatment could increase the expression of miR-149, suggesting a slight suppressive effect by JAKs on miR-149 expression in resting keratinocytes (Paper III Figure 4a). Altogether these results demonstrate that IFN-γ downregulates miR-149 expression by modulating JAK-STAT axis.

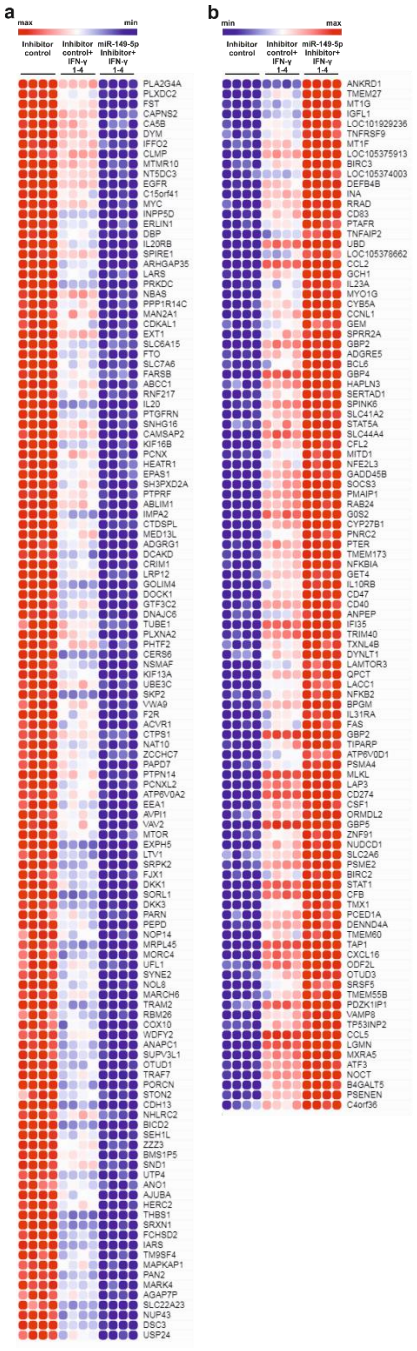
4.3.1.3 miR-149 regulates genes in JAK/STAT signalling pathway

Next, we aimed to test the effects of miR-149 on keratinocyte transcriptome. To do so, we inhibited endogenous miR-149 by transfecting miR-149 inhibitor in keratinocytes. Microarray analysis for whole transcriptome revealed that miR-149 inhibition had a robust effect on gene expression as 1164 genes (446 upregulated and 718 downregulated) were differentially expressed (Benjamini & Hochberg, $P < 0.05$, FCH > 1.4) compared to control oligonucleotides (Paper III Figure 5a). Interestingly, genes involved in JAK-STAT signalling pathway, MAPK signalling pathway, cytokine-cytokine receptor interaction, response to interferon-gamma and positive regulation of JAK-STAT cascade were enriched among the upregulated genes upon miR-149 inhibition in keratinocytes (Paper III Figure 5b-c). Our results indicate that miR-149 negatively regulates the JAK/STAT pathway in resting keratinocytes.

4.3.1.4 miR-149 potentiates IFN-γ responses

Next we analysed the effect of miR-149 on the keratinocyte transcriptome under inflammatory conditions in IFN-γ-treated keratinocytes. IFN-γ had a strong effect on the keratinocyte transcriptome and 4119 genes were found to be differentially expressed (Benjamini & Hochberg, $P < 0.05$, FCH > 1.4) (Paper III Supplementary Figure S2a). In order to explore the effects of miR-149 on keratinocyte responses towards IFN-γ, we analysed the transcriptome of keratinocytes where endogenous miR-149 was inhibited and cells were further stimulated with IFN-γ. Interestingly, miR-149 inhibition in IFN-γ-treated keratinocytes led to differential expression of 1077 genes compared to IFN-γ-treated keratinocytes transfected with control

oligonucleotide (Benjamini & Hochberg, P < 0.05, FCH > 1.4) (Paper III Supplementary Figure S3a).



c

KEGG pathway (deregulated genes)

Term	P-value	Adjusted P-value	Z-score
TNF signaling pathway_Homo sapiens_hsa04668	4.8E-06	0.001	-1.915
Cytokine-cytokine receptor interaction_Homo sapiens_hsa04060	1.2E-05	0.001	-1.853
Jak-STAT signaling pathway_Homo sapiens_hsa04630	8.6E-05	0.005	-1.880
Pathways in cancer_Homo sapiens_hsa05200	0.0007	0.014	-1.950
Herpes simplex infection_Homo sapiens_hsa05168	0.0003	0.012	-1.706
NOD-like receptor signaling pathway_Homo sapiens_hsa04621	0.0005	0.014	-1.650
Rheumatoid arthritis_Homo sapiens_hsa05323	0.0006	0.014	-1.632
NF-kappa B signaling pathway_Homo sapiens_hsa04064	0.0007	0.014	-1.568
Epithelial cell signaling in Helicobacter pylori infection_Homo sapiens_hsa05120	0.0011	0.018	-1.626
Ubiquitin mediated proteolysis_Homo sapiens_hsa04120	0.0010	0.018	-1.455
Toxoplasmosis_Homo sapiens_hsa05145	0.0023	0.036	-1.652
Small cell lung cancer_Homo sapiens_hsa05222	0.0030	0.042	-1.541

d

GO Biological processes (deregulated genes)

Term	P-value	Adjusted P-value	Z-score
cytokine-mediated signaling pathway (GO:0019221)	7.4E-08	0.0001	-1.346
cellular response to tumor necrosis factor (GO:0071356)	2.6E-06	0.002	-1.283
positive regulation of JAK-STAT cascade (GO:0046427)	9.1E-06	0.004	-1.487
response to lipopolysaccharide (GO:0032496)	1.3E-05	0.004	-1.711
tumor necrosis factor-mediated signaling pathway (GO:0033209)	1.3E-05	0.004	-1.274
response to interferon-gamma (GO:0034341)	1.4E-05	0.004	-1.299
cellular response to cytokine stimulus (GO:0071345)	2.1E-05	0.005	-1.164
positive regulation of tyrosine phosphorylation of STAT protein (GO:0042531)	3.4E-05	0.007	-1.556
positive regulation of extrinsic apoptotic signaling pathway via death domain receptors (GO:1902043)	4.9E-05	0.009	-3.245
positive regulation of cell migration (GO:0030335)	5.1E-05	0.009	-1.906
regulation of tyrosine phosphorylation of STAT protein (GO:0042509)	7.4E-05	0.010	-2.159
positive regulation of macrophage chemotaxis (GO:0010759)	7.8E-05	0.010	-3.271
positive regulation of macrophage migration (GO:1905523)	7.8E-05	0.010	-2.835
STAT cascade (GO:0097696)	0.0001	0.015	-1.912
response to molecule of bacterial origin (GO:0002237)	0.0001	0.016	-1.286
response to organic cyclic compound (GO:0014070)	0.0001	0.016	-1.357
regulation of smooth muscle cell proliferation (GO:0048660)	0.0002	0.016	-1.596
regulation of apoptotic process (GO:0042981)	0.0002	0.017	-1.764
response to cytokine (GO:0034097)	0.0002	0.018	-1.267
positive regulation of apoptotic process (GO:0043065)	0.0002	0.019	-1.666
positive regulation of cell motility (GO:2000147)	0.0002	0.019	-1.213
cellular response to increased oxygen levels (GO:0036295)	0.0003	0.023	-2.173
regulation of cell proliferation (GO:0042127)	0.0003	0.025	-1.114
protein insertion into membrane (GO:0051205)	0.0004	0.027	-2.563
regulation of mononuclear cell migration (GO:0071675)	0.0004	0.027	-2.357
regulation of granulocyte chemotaxis (GO:0071622)	0.0005	0.032	-2.422
inflammatory response (GO:0006954)	0.0007	0.044	-1.755
regulation of macrophage chemotaxis (GO:0010758)	0.0007	0.045	-2.521
protein deubiquitination (GO:0016579)	0.0008	0.047	-1.753

Figure 13: (a-b) IFN-γ-induced and suppressed genes whose expression was further potentiated upon miR-149 inhibition. (c-d) KEGG pathway and biological processes enriched among these genes.

Remarkably, miR-149 inhibition potentiated IFN-γ responses in keratinocytes and 126 IFN-γ-suppressed genes were further suppressed and 103 IFN-γ-induced genes were further induced upon miR-149 inhibition in keratinocytes (*Figure 13a-b*, Paper III Figure 6a-b). Strikingly, miR-149 strongly affected the genes regulating IFN-γ/JAK/STAT axis such as STAT1, STAT5A, SOCS3, IL20, IL23A, IL10RB, IL20RB, CCL5, CCL2 and CXCL16, indicating that miR-149 negatively regulates IFN-γ-induced inflammatory pathways (*Figure 13c-d*, Paper III, Figure 6c-d).

4.3.2 Discussion

Our results point to a negative regulation of IFN-γ/JAK/STAT axis in keratinocytes by miR-149. MiR-149 was identified as one of the downregulated miRNAs in psoriatic keratinocytes compared to normal keratinocytes. Decreased expression of miR-149 was not identified in previous studies profiling the miRNA signature of psoriasis. This could be due the fact that we analysed the landscape of miRNAs of sorted psoriatic keratinocytes while other studies have used mixture of cellular pool obtained from full depth skin biopsies (Joyce et al., 2011, Sonkoly et al., 2007, Zibert et al., 2010). Downregulation of miR-149 in psoriatic keratinocytes was further validated in an extend validation cohort using qRT-PCR. Functionally, miR-149 has been previously implicated in inflammation, apoptosis, proliferation, cancer metastasis and chemo-resistance (Chen et al., 2018, He et al., 2018, She et al., 2014, Tian and Yan, 2016, Wang et al., 2012, Xu et al., 2014). Our findings suggest an anti-inflammatory role of miR-149 in psoriasis which is in line with previous studies showing anti-inflammatory role of this miRNA in macrophages and osteoarthritis (Chen et al., 2018, Xu et al., 2014).

Among tested psoriasis-associated cytokines only IFN-γ was able to regulate miR-149 expression in human primary keratinocytes. IFN-γ is involved in psoriasis pathogenesis and mRNA expression of this cytokine has been shown to be upregulated in psoriasis skin along with increased levels of IFN-γ in serum of psoriasis patients (Harden et al., 2015, Johnson-

Huang et al., 2012, Nestle et al., 2009b, Perera et al., 2012). Single treatment of IFN-γ in human primary keratinocytes resulted in rapid transcriptional decrease of primary transcript of miR-149 at 1 hour and a strong suppression of mature miR-149 was observed at 24 hour which remained suppressed even 4 day after IFN-γ treatment. These results indicate that upregulated IFN-γ levels in psoriasis skin (Johnson-Huang et al., 2012, Nestle et al., 2009b, Perera et al., 2012) could at least in part be responsible for miR-149 downregulation.

Mechanistically, IFN-γ-induced JAK/STAT axis was important in regulating miR-149 expression as inhibition of JAKs rescued IFN-γ-suppressed miR-149 expression. Inhibition of JAKs in resting keratinocytes, induced miR-149 expression, suggesting JAK/STAT axis act as a block on miR-149 expression even is homeostatic condition.

Transcriptomic analysis upon miR-149 inhibition in resting keratinocyte revealed that miR-149 may act as a break on inflammatory mediators as the genes regulating immune-related functions and biological pathways such as JAK-STAT signalling were significantly upregulated. Remarkably, 57% of these upregulated genes were IFN-γ-inducible genes and 53% of downregulated genes were IFN-γ-suppressed genes. These results hint towards a global impact of miR-149 on IFN-γ/JAK/STAT pathway in homeostatic conditions.

Notably, inhibition of miR-149 in IFN-γ-treated keratinocytes further potentiated IFN-γ responses and the expression of JAK-STAT signalling pathway genes such as STAT1, STAT5A, SOCS3, IL20, IL23A, IL10RB, IL20RB, CCL5, CCL2 and CXCL16 were further induced in these cells. Our results suggest that miR-149 may negatively regulate IFN-γ/JAK/STAT axis by targeting one or more regulators of this pathway.

JAK/STAT signalling is active in psoriasis (Hald et al., 2013) and has been targeted by JAK inhibitors such as tofacitinib (JAK1/3), ruxolitinib (JAK1/2) and baricitinib (JAK1/2) (Palanivel et al., 2014, Welsch et al., 2017). Recently, a study demonstrated that STAT1 mRNA is upregulated and both the phosphorylation sites of STAT1 (p-STAT1-Tyr701 and p-STAT1-

Ser727) are phosphorylated in psoriasis skin lesions (Hald et al., 2013). STAT1 is key transcription factor in regulating IFN-γ responses by binding to the interferon gamma activated sequence (GAS) promoter element to induce expression of inflammatory mediators such as CXCL9, 10, 11 and IL-6 (Kanda et al., 2007, Ramana et al., 2002). CXCL 9, 10, 11 and IL-6 are also present in high levels in psoriasis skin and are involved in either T_H1 cell chemotaxis or inflammation (Nedoszytko et al., 2014, Turner et al., 2014). ELISA and qRT-PCR analysis showed that miR-149 negatively regulated the secretion and expression of basal as well as IFN-γ-induced IL-6 in keratinocytes. IL-6 has been shown to be a direct target of miR-149 in other cell type (Li et al., 2015). In addition, to IL-6 which has been shown to be direct target, other IFN-γ-response genes (CXCL 9, 10, 11) were regulated by miR-149, suggesting that one or more mediators of this pathway are targeted by miR-149.These results further provide evidence for negative regulation of keratinocytes response to IFN-γ by miR-149.

In conclusion, our results indicate an anti-inflammatory role of miR-149 in psoriasis specifically in the context of JAK/STAT signalling. Downregulation of miR-149 in psoriatic keratinocytes could lead to activation of JAK/STAT signalling pathway leading to the chronic inflammation. To exert its anti-inflammatory function it is plausible that miR-149 targets one or more elements of the IFN-γ/JAK1-2/STAT1 pathway.

4.4 EFFECTS OF TOFACITINIB ON KERATINOCYTE TRANSCRIPTOME

In recent years, JAK inhibitors gained popularity as an immunomodulatory drug to treat a variety of diseases such as psoriasis, atopic dermatitis, alopecia areata and vitiligo (Palanivel et al., 2014, Welsch et al., 2017). Tofacitinib targets JAK1 and JAK3 and to a lesser extent to JAK2 and Tyk2. Therapeutically, tofacitinib has been shown efficacious in treating psoriasis in oral and topical formulations (phase III and phase II trials) (Palanivel et al., 2014, Welsch et al., 2017). Effects of tofacitinib were thought to be mediated by T cells, however evidence suggest that STAT3 pathway is also active in keratinocytes (Hsu and Armstrong, 2014). In this study we aimed to investigate if the targets of tofacitinib are expressed by keratinocytes and the possible effects of tofacitinib on keratinocytes transcriptome.

4.4.1 Results

4.4.1.1 Keratinocytes expresses JAK proteins

First to test that whether the targets of tofacitinib- JAKs (JAK1, JAK2, JAK3 and Tyk2) are expressed by keratinocytes we performed qRT-PCR analysis for JAK1, JAK2, JAK3 and Tyk2 in comparison to PBMCs. Expression analysis verified that the JAK1 JAK2 and Tyk2 were expressed by keratinocytes and their expression was comparable to PBMCs, while JAK3 was expressed at a lower level (Paper IV, Figure 1a). Western blot analysis for JAK1, JAK2 and JAK3 using cell lysate from cultured human primary keratinocytes also confirmed their protein expression in keratinocytes (Paper IV, Figure 1b).

4.4.1.2 Tofacitinib inhibits JAK-STAT signalling in keratinocytes

Next we tested whether tofacitinib can inhibit JAK-STAT signalling in keratinocytes. To this end, keratinocytes were pre-treated with different dose of tofacitinib or vehicle control DMSO

followed by treatment with IL-22, a known activator of JAK-STAT signalling. qRT-PCR analysis of downstream genes of IL-22/STAT3 signalling- S100A8 and S100A9 verified that the pre-treatment with all the tested doses of tofacitinib were able to block IL-22 induced expression of S100A8 and S100A9 in keratinocytes (Paper IV, Figure 2a-b). Moreover, western blot analysis of p-STAT3 (Tyr705 and Ser727) and p-STAT1 further confirmed that tofacitinib prevented the IL-22 induced phosphorylation of STAT3 and STAT1 leading to inhibition of JAK-STAT axis in keratinocytes (Paper IV, Figure 3a-c).

4.4.1.3 Tofacitinib prevents IL-22 induced gene expression changes

In order to observe effects of tofacitinib on the keratinocyte transcriptome, we performed whole transcriptome analysis using microarray. A single treatment of IL-22 in keratinocytes resulted in the differential expression 898 genes at 6 hour after the treatment. Strikingly, 93% of the Il-22-regulated gene expression changes were partially prevented by pre-treatment with tofacitinib. Out of these differentially expressed genes 193 genes were significantly differentially expressed by both IL-22 vs untreated keratinocytes and tofacitinib pre-treated + IL-22 treated vs IL-22 treated keratinocytes (*Figure 14a*, Paper IV, Figure 4a). Interestingly, all the IL-22-regulated genes were regulated in opposite direction by tofacitinib pre-treatment (*Figure 14a*, Paper IV, Figure 4a). Network analysis among these differentially expressed genes further confirmed a direct effect of tofacitinib on JAK-STAT axis in keratinocytes as the genes regulating JAK-STAT pathway along with T-cell activation, endothelial and epidermal cell differentiation were clustered together (*Figure 14b*, Paper IV, Figure 4b). Moreover, psoriasis- associated biological processes such as keratinocyte differentiation and response to cytokines were significantly enriched among the differentially expressed genes (data not shown). The enriched biological processes among tofacitinib regulated genes corresponds to the known IL-22 functions in psoriasis such as inhibition of terminal differentiation of

keratinocytes and regulation of keratinocyte proliferation and suggest that tofacitinib could prevent these IL-22-induced effects.

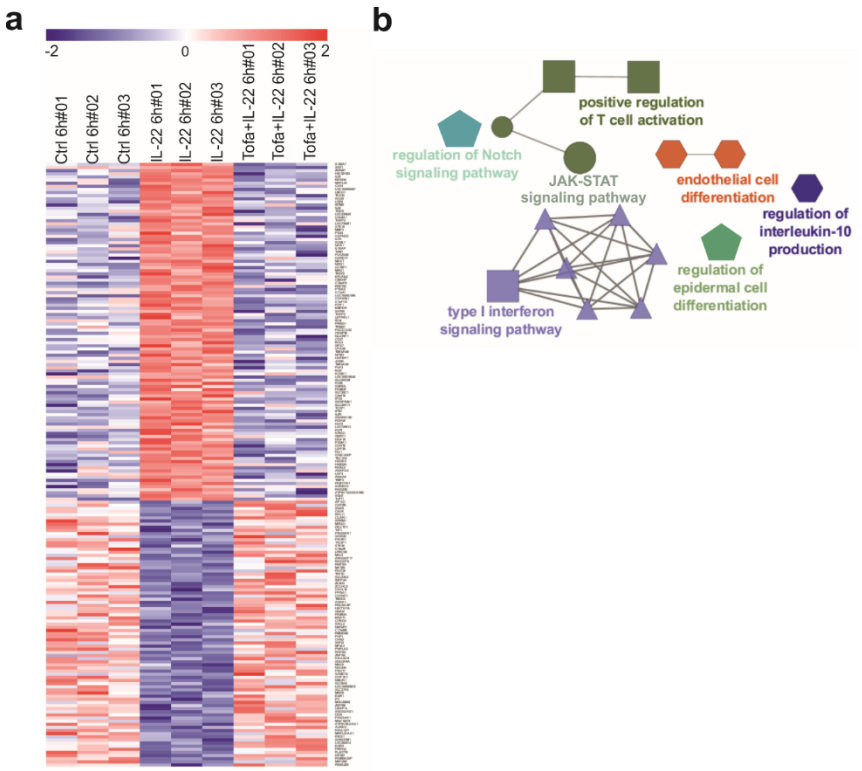


Figure 14: (a) IL-22- regulated genes which were regulated by tofacitinib in opposite direction. (b) Network analysis among differentially expressed genes. Reproduced with permission from Srivastava et al., 2018. Copyright Acta Dermato-Venereologica.

Next we validated the expression of two IL-22-regulated genes S100A7 and EGR1 which were regulated by tofacitinib in opposite direction. S100A7 (psoriasin) is a psoriasis associated antimicrobial peptide which is upregulated in psoriasis and regulates keratinocytes proliferation and differentiation. EGR1 is a transcription factor regulating cell growth, differentiation and survival. Expression analysis using qRT-PCR confirmed upregulation of S100A7 and downregulation of EGR1 by IL-22 in keratinocytes and regulated by tofacitinib in opposite direction (*Figure 15a-b*, Paper IV, Figure 5a-b). These results indicate that tofacitinib could reverse IL-22 effects in keratinocytes.

Next, to verify the relevance of these findings in psoriasis we analysed the expression of S100A7 and EGR1 by qRT-PCR in $CD45^{neg}$ cells (predominantly keratinocytes) isolated from

healthy and psoriasis (lesional and non-lesional) skin. Expression of S100A7 was found upregulated in psoriatic keratinocytes, which is in line with our results and previous findings (*Figure 15c,* Paper IV, Figure 5c). On contrary, EGR1 was downregulated in psoriatic keratinocytes compared to keratinocytes isolated from healthy donors (*Figure 15d,* Paper IV, Figure 5d).

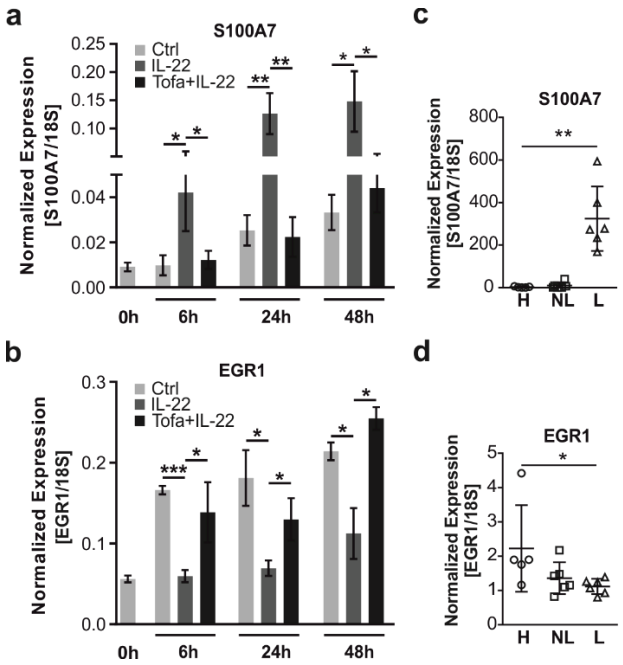


Figure 15: (a-b) Expression of S100A7 and EGR1 in keratinocytes upon IL-22 or tofacitinib treatment. (c-d) Expression of S100A7 and EGR1 in keratinocytes sorted from healthy or psoriasis (lesional and non-lesional) skin. . Reproduced with permission from Srivastava et al., 2018. Copyright Acta Dermato-Venereologica.

4.4.2 Discussion

Altogether, our results show that tofacitinib have direct effects on keratinocytes transcriptome and can reverse IL-22-mediated gene expression changes by modulating JAK-STAT axis. These results imply that the effects of tofacitinib are not limited only to T-cells but can also be mediated by keratinocytes. JAK-STAT axis is important in psoriasis, as evidenced by the genetic association of STAT3 and Tyk2 with psoriasis (Tsoi et al., 2012), the role of STAT3 in regulating cytokine (IL-6, IL-10, IL-20 and IL-22) signalling and T_H17 cell differentiation (Miyoshi et al., 2011, Sestito et al., 2011). In addition, keratinocyte-specific constitutive

activation of STAT3 in mice resulted in spontaneous development of psoriasis-like skin inflammation (Sano et al., 2005).

Targeting JAK-STAT axis in keratinocytes could partially explain the effect of topical tofacitinib treatment in a phase II randomized clinical trial of psoriasis and atopic dermatitis patients (Bissonnette et al., 2016).Our findings are in line with a recent study showing a robust reduction in the keratinocyte-meditated transcriptomic changes in lesional skin of psoriasis patients treated with oral tofacitinib (Krueger et al., 2016). Our findings support a mode of action where tofacitinib could target both keratinocytes and T-cells, which could be relevant for both topical and oral formulations.

In summary, tofacitinib blocks the IL-22-mediated JAK-STAT signalling by targeting JAK1 and Tyk2 (*Figure 16*) in keratinocytes and reverses IL-22 mediated transcriptomic changes. In the context of psoriasis this mechanism could prevent epidermal hyperplasia and papilomatosis induced by IL-22 (*Figure 16*).

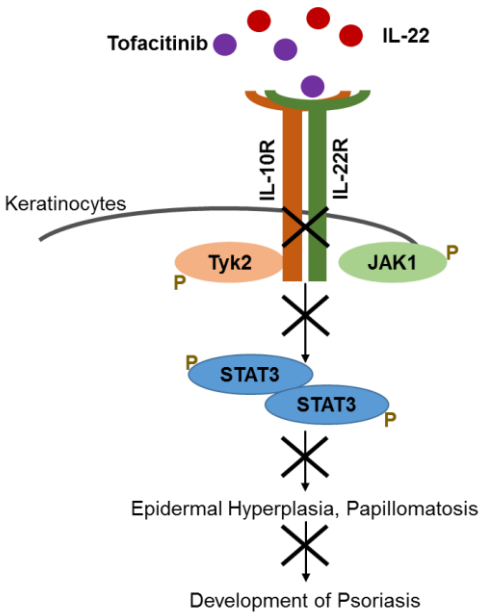


Figure 16: Schematic of mode of action of tofacitinib in keratinocytes.

5 CONCLUSIONS

Communication of keratinocytes with trafficking immune cells and skin resident cells has been shown to be critical in psoriasis. Cytokine and chemokine networks shape this crosstalk and regulate inflammatory pathways in psoriasis. In particular, keratinocyte-derived inflammatory mediators and chemokine attract trafficking immune cells and induce cellular inflammation to maintain auto loop of inflammation. The objective of this thesis is to uncover the functional and regulatory role of miRNAs in regulating keratinocytes response to different cytokines in psoriasis.

In paper I, we show a protective association of miR-146a (rs2910164-CC genotype) to earlier onset of psoriasis in *HLA-C*06*-negative patients. In addition, miR-146 was found to be a potent suppressor of IL-17A-mediated skin inflammation and negatively regulated the inflammatory mediators, such as IL-8 and CCL20. In line with our findings in human keratinocytes, an earlier onset of imiqimod-induced psoriasiform skin inflammation was observed in miR-146a knockout mice compared to WT mice. Interestingly lack of miR-146a inhibited spontaneous resolution of the psoriasis-like skin inflammation. Remarkably, miR-146a was also found to have therapeutic potential as injection with miR-146a alleviates psoriasis-like skin inflammation in mice. Future studies may target the therapeutic potential of miR-146a in resolving skin inflammation in psoriasis.

In Paper II we identify keratinocyte-specific alterations in miRNA expression in psoriasis. Here, we show several yet not identified and characterized miRNAs in psoriasis whose expression was altered in a keratinocyte. Deregulated expression of human-specific miR-941 was validated with qRT-PCR along with miR-1307-3p. This study provide a platform for future studies focusing to characterize the functional role of miRNAs in psoriasis.

In paper III we expanded our observation from paper II. We identified miR-149 as a downregulated miRNA in psoriatic keratinocytes. Our *in vitro* regulation experiment hinted that the high levels of IFN-γ in psoriasis could partially contribute to the downregulation of miR-149. JAK/STAT axis was pivotal in regulating miR-149 expression in response to IFN-γ as well as in resting keratinocytes. Functionally, miR-149 negatively regulated the JAK/STAT pathway and inflammatory mediators such as CXCL9, 10, 11 and IL-6. These results suggest that downregulation of miR-149 in psoriatic keratinocytes could potentiate IFN-γ-mediated responses in psoriasis. These results would be further corroborated by establishing the mechanism of negative regulation of inflammation by miR-149. In addition animal models of psoriasis would be utilized to verify the functional role of miR-149 (injection of miR-149 mimic and inhibitor) in regulating psoriasis pathogenesis *in vivo*.

Paper IV identified a new mechanism of the immunomodulatory JAK inhibitor- tofacitinib. Here, we show that keratinocytes express the potential targets of tofacitinib and that JAK/STAT signalling was suppressed by tofacitinib in keratinocytes. Molecularly, tofacitinb has a direct and robust effect on keratinocyte transcriptome and rescued the IL-22-regulated transcriptomic changes. This mechanism could in part be responsible for the success of topical tofacitinib formulations in psoriasis and atopic dermatitis.

Altogether, we have explored the function of two miRNAs, miR-146a and miR-149, in regulating cytokine signalling in psoriasis. In addition, we also described the keratinocyte-specific changes in miRNAs expression in psoriasis. Finally, we outlined that tofacitinib may also target keratinocytes to mediate its effect in treating psoriasis. In conclusion, this thesis highlighted the functional role of miRNAs in shaping the cytokine response in keratinocytes and investigated their potential role in psoriasis pathogenesis, progression and maintenance.

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7 REFERENCES

Abdallah MA, Abdel-Hamid MF, Kotb AM, Mabrouk EA. Serum interferon-gamma is a psoriasis severity and prognostic marker. Cutis 2009;84(3):163-8.

Abtin A, Jain R, Mitchell AJ, Roediger B, Brzoska AJ, Tikoo S, et al. Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection. Nat Immunol 2014;15(1):45-53.

Albanesi C, Scarponi C, Giustizieri ML, Girolomoni G. Keratinocytes in inflammatory skin diseases. Curr Drug Targets Inflamm Allergy 2005;4(3):329-34.

Albanesi C, Scarponi C, Sebastiani S, Cavani A, Federici M, Sozzani S, et al. A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. J Leukoc Biol 2001;70(4):617-23.

Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013;14(8):475-88.

Arakawa A, Siewert K, Stohr J, Besgen P, Kim SM, Ruhl G, et al. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med 2015;212(13):2203-12.

Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 2005;122(4):553-63.

Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116(2):281-97.

Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136(2):215-33.

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, et al. Dicer is essential for mouse development. Nat Genet 2003;35(3):215-7.

Bieber T. The pro- and anti-inflammatory properties of human antigen-presenting cells expressing the high affinity receptor for IgE (Fc epsilon RI). Immunobiology 2007;212(6):499-503.

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 2009;25(8):1091-3.

Bissonnette R, Papp KA, Poulin Y, Gooderham M, Raman M, Mallbris L, et al. Topical tofacitinib for atopic dermatitis: a phase IIa randomized trial. Br J Dermatol 2016;175(5):902-11.

Blanpain C, Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol 2009;10(3):207-17.

Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med 2011;208(6):1189-201.

Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. J Immunol 2005;174(6):3695-702.

Botchkarev VA. Integration of the Transcription Factor-Regulated and Epigenetic Mechanisms in the Control of Keratinocyte Differentiation. J Investig Dermatol Symp Proc 2015;17(2):30-2.

Boyman O, Hefti HP, Conrad C, Nickoloff BJ, Suter M, Nestle FO. Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha. J Exp Med 2004;199(5):731-6.

Brandrup F, Hauge M, Henningsen K, Eriksen B. Psoriasis in an unselected series of twins. Arch Dermatol 1978;114(6):874-8.

Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6(11):857-66.

Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6(4):328-40.

Capon F, Novelli G, Semprini S, Clementi M, Nudo M, Vultaggio P, et al. Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. J Invest Dermatol 1999a;112(1):32-5.

Capon F, Semprini S, Dallapiccola B, Novelli G. Evidence for interaction between psoriasis-susceptibility loci on chromosomes 6p21 and 1q21. Am J Hum Genet 1999b;65(6):1798-800.

Chan JR, Blumenschein W, Murphy E, Diveu C, Wiekowski M, Abbondanzo S, et al. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. J Exp Med 2006;203(12):2577-87.

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 2013;14:128.

Chen Q, Wu S, Wu Y, Chen L, Pang Q. MiR-149 suppresses the inflammatory response of chondrocytes in osteoarthritis by down-regulating the activation of TAK1/NF-kappaB. Biomed Pharmacother 2018;101:763-8.

Cheuk S, Schlums H, Gallais Serezal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8(+) T Cells Poised for Cytotoxic Function in Human Skin. Immunity 2017;46(2):287-300.

Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, et al. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med 2016;213(11):2399-412.

Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K, Dowgiert RK, et al. The vast majority of CLA+ T cells are resident in normal skin. J Immunol 2006;176(7):4431-9.

Davis-Dusenbery BN, Hata A. Mechanisms of control of microRNA biogenesis. J Biochem 2010;148(4):381-92.

de Cid R, Riveira-Munoz E, Zeeuwen PL, Robarge J, Liao W, Dannhauser EN, et al. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. Nat Genet 2009;41(2):211-5.

Debets R, Hegmans JP, Croughs P, Troost RJ, Prins JB, Benner R, et al. The IL-1 system in psoriatic skin: IL-1 antagonist sphere of influence in lesional psoriatic epidermis. J Immunol 1997;158(6):2955-63.

Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature 2004;432(7014):231-5.

Desruisseaux MS, Nagajyothi, Trujillo ME, Tanowitz HB, Scherer PE. Adipocyte, adipose tissue, and infectious disease. Infect Immun 2007;75(3):1066-78.

Di Meglio P, Nestle FO. The role of IL-23 in the immunopathogenesis of psoriasis. F1000 Biol Rep 2010;2.

Di Meglio P, Perera GK, Nestle FO. The multitasking organ: recent insights into skin immune function. Immunity 2011;35(6):857-69.

Duffy DL, Spelman LS, Martin NG. Psoriasis in Australian twins. J Am Acad Dermatol 1993;29(3):428-34.

Elder JT. The Quest for Psoriasis Autoantigens: Genetics Meets Immunology in the Melanocyte. J Invest Dermatol 2017;137(10):2042-5.

Elder JT, Nair RP, Guo SW, Henseler T, Christophers E, Voorhees JJ. The genetics of psoriasis. Arch Dermatol 1994;130(2):216-24.

Elmen J, Lindow M, Silahtaroglu A, Bak M, Christensen M, Lind-Thomsen A, et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 2008;36(4):1153-62.

Farber EM, Nall ML. The natural history of psoriasis in 5,600 patients. Dermatologica 1974;148(1):1-18.

Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. Arch Dermatol 1974;109(2):207-11.

Forman JJ, Coller HA. The code within the code: microRNAs target coding regions. Cell Cycle 2010;9(8):1533-41.

Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19(1):92-105.

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet 2002;3(3):199-209.

Gaur U, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem Pharmacol 2003;66(8):1403-8.

Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol 2008;8(8):594-606.

Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. Curr Opin Immunol 2008;20(4):401-7.

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, et al. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 2006;312(5770):75-9.

Girolomoni G, Griffiths CE, Krueger J, Nestle FO, Nicolas JF, Prinz JC, et al. Early intervention in psoriasis and immune-mediated inflammatory diseases: A hypothesis paper. J Dermatolog Treat 2015;26(2):103-12.

Girolomoni G, Strohal R, Puig L, Bachelez H, Barker J, Boehncke WH, et al. The role of IL-23 and the IL-23/TH 17 immune axis in the pathogenesis and treatment of psoriasis. J Eur Acad Dermatol Venereol 2017;31(10):1616-26.

Goldminz AM, Au SC, Kim N, Gottlieb AB, Lizzul PF. NF-kappaB: an essential transcription factor in psoriasis. J Dermatol Sci 2013;69(2):89-94.

Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004;432(7014):235-40.

Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell 2007;27(1):91-105.

Gudjonsson JE, Johnston A, Dyson M, Valdimarsson H, Elder JT. Mouse models of psoriasis. J Invest Dermatol 2007;127(6):1292-308.

Guinea-Viniegra J, Jimenez M, Schonthaler HB, Navarro R, Delgado Y, Concha-Garzon MJ, et al. Targeting miR-21 to treat psoriasis. Sci Transl Med 2014;6(225):225re1.

Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nograles KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J Immunol 2008;181(10):7420-7.

Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15(8):509-24.

Hald A, Andres RM, Salskov-Iversen ML, Kjellerup RB, Iversen L, Johansen C. STAT1 expression and activation is increased in lesional psoriatic skin. Br J Dermatol 2013;168(2):302-10.

Harden JL, Johnson-Huang LM, Chamian MF, Lee E, Pearce T, Leonardi CL, et al. Humanized anti-IFN-gamma (HuZAF) in the treatment of psoriasis. J Allergy Clin Immunol 2015;135(2):553-6.

Harper EG, Guo C, Rizzo H, Lillis JV, Kurtz SE, Skorcheva I, et al. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. J Invest Dermatol 2009;129(9):2175-83.

Hartupee J, Liu C, Novotny M, Li X, Hamilton T. IL-17 enhances chemokine gene expression through mRNA stabilization. J Immunol 2007;179(6):4135-41.

Hassan-Zahraee M, Wu J, Gordon J. Rapid synthesis of IFN-gamma by T cells in skin may play a pivotal role in the human skin immune system. Int Immunol 1998;10(11):1599-612.

Hausser J, Zavolan M. Identification and consequences of miRNA-target interactions--beyond repression of gene expression. Nat Rev Genet 2014;15(9):599-612.

He Y, Yu D, Zhu L, Zhong S, Zhao J, Tang J. miR-149 in Human Cancer: A Systemic Review. J Cancer 2018;9(2):375-88.

Hermann H, Runnel T, Aab A, Baurecht H, Rodriguez E, Magilnick N, et al. miR-146b Probably Assists miRNA-146a in the Suppression of Keratinocyte Proliferation and Inflammatory Responses in Psoriasis. J Invest Dermatol 2017;137(9):1945-54.

Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D3. Use of calcitriol (1,25-dihydroxyvitamin D3) for treatment of psoriasis. Arch Dermatol 1987;123(12):1677-83a.

Howe EA, Sinha R, Schlauch D, Quackenbush J. RNA-Seq analysis in MeV. Bioinformatics 2011;27(22):3209-10.

Hsu L, Armstrong AW. JAK inhibitors: treatment efficacy and safety profile in patients with psoriasis. J Immunol Res 2014;2014:283617.

Hueber W, Patel DD, Dryja T, Wright AM, Koroleva I, Bruin G, et al. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. Sci Transl Med 2010;2(52):52ra72.

Hydbring P, Badalian-Very G. Clinical applications of microRNAs. F1000Res 2013;2:136.

Javitz HS, Ward MM, Farber E, Nail L, Vallow SG. The direct cost of care for psoriasis and psoriatic arthritis in the United States. J Am Acad Dermatol 2002;46(6):850-60.

Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2008;105(20):7269-74.

Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 2011;332(6035):1284-8.

Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, et al. Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 2005;120(5):623-34.

Johnson-Huang LM, Suarez-Farinas M, Pierson KC, Fuentes-Duculan J, Cueto I, Lentini T, et al. A single intradermal injection of IFN-gamma induces an inflammatory state in both non-lesional psoriatic and healthy skin. J Invest Dermatol 2012;132(4):1177-87.

Johnston A, Fritz Y, Dawes SM, Diaconu D, Al-Attar PM, Guzman AM, et al. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. J Immunol 2013;190(5):2252-62.

Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 2015;16(7):421-33.

Joyce CE, Zhou X, Xia J, Ryan C, Thrash B, Menter A, et al. Deep sequencing of small RNAs from human skin reveals major alterations in the psoriasis miRNAome. Hum Mol Genet 2011;20(20):4025-40.

Kagami S, Rizzo HL, Lee JJ, Koguchi Y, Blauvelt A. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. J Invest Dermatol 2010;130(5):1373-83.

Kanda N, Shimizu T, Tada Y, Watanabe S. IL-18 enhances IFN-gamma-induced production of CXCL9, CXCL10, and CXCL11 in human keratinocytes. Eur J Immunol 2007;37(2):338-50.

Kim J, Krueger JG. The immunopathogenesis of psoriasis. Dermatol Clin 2015;33(1):13-23.

Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11(9):597-610.

Krueger J, Clark JD, Suarez-Farinas M, Fuentes-Duculan J, Cueto I, Wang CQ, et al. Tofacitinib attenuates pathologic immune pathways in patients with psoriasis: A randomized phase 2 study. J Allergy Clin Immunol 2016;137(4):1079-90.

Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44(W1):W90-7.

Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 2009;30(3):131-41.

Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, et al. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014;5:5621.

Leaman D, Chen PY, Fak J, Yalcin A, Pearce M, Unnerstall U, et al. Antisense-mediated depletion reveals essential and specific functions of microRNAs in Drosophila development. Cell 2005;121(7):1097-108.

Lebre MC, van der Aar AM, van Baarsen L, van Capel TM, Schuitemaker JH, Kapsenberg ML, et al. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. J Invest Dermatol 2007;127(2):331-41.

Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75(5):843-54.

Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004;23(20):4051-60.

Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, Aberdam D, Knight RA, Melino G, et al. miR-203 represses 'stemness' by repressing DeltaNp63. Cell Death Differ 2008;15(7):1187-95.

Lerman G, Avivi C, Mardoukh C, Barzilai A, Tessone A, Gradus B, et al. MiRNA expression in psoriatic skin: reciprocal regulation of hsa-miR-99a and IGF-1R. PLoS One 2011;6(6):e20916.

Li H, Yao Q, Mariscal AG, Wu X, Hulse J, Pedersen E, et al. Epigenetic control of IL-23 expression in keratinocytes is important for chronic skin inflammation. Nat Commun 2018;9(1):1420.

Li P, Shan JX, Chen XH, Zhang D, Su LP, Huang XY, et al. Epigenetic silencing of microRNA-149 in cancer-associated fibroblasts mediates prostaglandin E2/interleukin-6 signaling in the tumor microenvironment. Cell Res 2015;25(5):588-603.

Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov 2014;13(8):622-38.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433(7027):769-73.

Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 2001;104(4):487-501.

Lohcharoenkal W, Harada M, Loven J, Meisgen F, Landen NX, Zhang L, et al. MicroRNA-203 Inversely Correlates with Differentiation Grade, Targets c-MYC, and Functions as a Tumor Suppressor in cSCC. J Invest Dermatol 2016;136(12):2485-94.

Lovendorf MB, Mitsui H, Zibert JR, Ropke MA, Hafner M, Dyring-Andersen B, et al. Laser capture microdissection followed by next-generation sequencing identifies disease-related microRNAs in psoriatic skin that reflect systemic microRNA changes in psoriasis. Exp Dermatol 2015;24(3):187-93.

Lovendorf MB, Skov L. miRNAs in inflammatory skin diseases and their clinical implications. Expert Rev Clin Immunol 2015;11(4):467-77.

Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature 2007;445(7130):866-73.

Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci U S A 2005;102(52):19057-62.

Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol 2008;128(5):1207-11.

Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol 2014;32:227-55.

Mack JA, Anand S, Maytin EV. Proliferation and cornification during development of the mammalian epidermis. Birth Defects Res C Embryo Today 2005;75(4):314-29.

Martin DA, Towne JE, Kricorian G, Klekotka P, Gudjonsson JE, Krueger JG, et al. The emerging role of IL-17 in the pathogenesis of psoriasis: preclinical and clinical findings. J Invest Dermatol 2013;133(1):17-26.

Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol 2009;27:229-65.

McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol 2009;10(3):314-24.

Mehta A, Baltimore D. MicroRNAs as regulatory elements in immune system logic. Nat Rev Immunol 2016;16(5):279-94.

Meisgen F, Xu Landen N, Wang A, Rethi B, Bouez C, Zuccolo M, et al. MiR-146a negatively regulates TLR2-induced inflammatory responses in keratinocytes. J Invest Dermatol 2014;134(7):1931-40.

Meisgen F, Xu N, Wei T, Janson PC, Obad S, Broom O, et al. MiR-21 is up-regulated in psoriasis and suppresses T cell apoptosis. Exp Dermatol 2012;21(4):312-4.

Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell 2012;148(6):1172-87.

Miyoshi K, Takaishi M, Nakajima K, Ikeda M, Kanda T, Tarutani M, et al. Stat3 as a therapeutic target for the treatment of psoriasis: a clinical feasibility study with STA-21, a Stat3 inhibitor. J Invest Dermatol 2011;131(1):108-17.

Monteys AM, Spengler RM, Wan J, Tecedor L, Lennox KA, Xing Y, et al. Structure and activity of putative intronic miRNA promoters. RNA 2010;16(3):495-505.

Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 2011;124(14):1537-47.

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34(3):267-73.

Murphy JE, Robert C, Kupper TS. Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J Invest Dermatol 2000;114(3):602-8.

Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. Nat Genet 2009;41(2):199-204.

Naldi L. Epidemiology of psoriasis. Curr Drug Targets Inflamm Allergy 2004;3(2):121-8.

Nedoszytko B, Sokolowska-Wojdylo M, Ruckemann-Dziurdzinska K, Roszkiewicz J, Nowicki RJ. Chemokines and cytokines network in the pathogenesis of the inflammatory skin diseases: atopic dermatitis, psoriasis and skin mastocytosis. Postepy Dermatol Alergol 2014;31(2):84-91.

Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. Nat Rev Immunol 2009a;9(10):679-91.

Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med 2009b;361(5):496-509.

Nestle FO, Nickoloff BJ. From classical mouse models of psoriasis to a spontaneous xenograft model featuring use of AGR mice. Ernst Schering Res Found Workshop 2005(50):203-12.

Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suarez-Farinas M, Cardinale I, et al. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol 2008;159(5):1092-102.

Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, et al. A high-resolution structure of the pre-microRNA nuclear export machinery. Science 2009;326(5957):1275-9.

Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, et al. Chromatin structure analyses identify miRNA promoters. Genes Dev 2008;22(22):3172-83.

Palanivel JA, Macbeth AE, Chetty NC, Levell NJ. An insight into JAK-STAT signalling in dermatology. Clin Exp Dermatol 2014;39(4):513-8.

Pasparakis M, Courtois G, Hafner M, Schmidt-Supprian M, Nenci A, Toksoy A, et al. TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature 2002;417(6891):861-6.

Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol 2014;14(5):289-301.

Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000;408(6808):86-9.

Perera GK, Di Meglio P, Nestle FO. Psoriasis. Annu Rev Pathol 2012;7:385-422.

Piskin G, Sylva-Steenland RM, Bos JD, Teunissen MB. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. J Immunol 2006;176(3):1908-15.

Pivarcsi A, Bodai L, Rethi B, Kenderessy-Szabo A, Koreck A, Szell M, et al. Expression and function of Toll-like receptors 2 and 4 in human keratinocytes. Int Immunol 2003;15(6):721-30.

Pivarcsi A, Stahle M, Sonkoly E. Genetic polymorphisms altering microRNA activity in psoriasis--a key to solve the puzzle of missing heritability? Exp Dermatol 2014;23(9):620-4.

Prinz J, Braun-Falco O, Meurer M, Daddona P, Reiter C, Rieber P, et al. Chimaeric CD4 monoclonal antibody in treatment of generalised pustular psoriasis. Lancet 1991;338(8762):320-1.

Ramana CV, Gil MP, Schreiber RD, Stark GR. Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trends Immunol 2002;23(2):96-101.

Ramirez-Carrozzi V, Sambandam A, Luis E, Lin Z, Jeet S, Lesch J, et al. IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. Nat Immunol 2011;12(12):1159-66.

Ramnath D, Tunny K, Hohenhaus DM, Pitts CM, Bergot AS, Hogarth PM, et al. TLR3 drives IRF6-dependent IL-23p19 expression and p19/EBI3 heterodimer formation in keratinocytes. Immunol Cell Biol 2015;93(9):771-9.

Rapp SR, Feldman SR, Exum ML, Fleischer AB, Jr., Reboussin DM. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol 1999;41(3 Pt 1):401-7.

Ray-Jones H, Eyre S, Barton A, Warren RB. One SNP at a Time: Moving beyond GWAS in Psoriasis. J Invest Dermatol 2016;136(3):567-73.

Rebane A, Runnel T, Aab A, Maslovskaja J, Ruckert B, Zimmermann M, et al. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. J Allergy Clin Immunol 2014;134(4):836-47 e11.

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 2000;403(6772):901-6.

Rennekampff HO, Hansbrough JF, Kiessig V, Dore C, Sticherling M, Schroder JM. Bioactive interleukin-8 is expressed in wounds and enhances wound healing. J Surg Res 2000;93(1):41-54.

Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017;16(3):203-22.

Russell TJ, Schultes LM, Kuban DJ. Histocompatibility (HL-A) antigens associated with psoriasis. N Engl J Med 1972;287(15):738-40.

Sabat R, Ouyang W, Wolk K. Therapeutic opportunities of the IL-22-IL-22R1 system. Nat Rev Drug Discov 2014;13(1):21-38.

Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, Kiguchi K, et al. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. Nat Med 2005;11(1):43-9.

Savage LJ, Wittmann M, McGonagle D, Helliwell PS. Ustekinumab in the Treatment of Psoriasis and Psoriatic Arthritis. Rheumatol Ther 2015;2(1):1-16.

Schwandner R, Yamaguchi K, Cao Z. Requirement of tumor necrosis factor receptor-associated factor (TRAF)6 in interleukin 17 signal transduction. J Exp Med 2000;191(7):1233-40.

Sestito R, Madonna S, Scarponi C, Cianfarani F, Failla CM, Cavani A, et al. STAT3-dependent effects of IL-22 in human keratinocytes are counterregulated by sirtuin 1 through a direct inhibition of STAT3 acetylation. FASEB J 2011;25(3):916-27.

She X, Yu Z, Cui Y, Lei Q, Wang Z, Xu G, et al. miR-128 and miR-149 enhance the chemosensitivity of temozolomide by Rap1B-mediated cytoskeletal remodeling in glioblastoma. Oncol Rep 2014;32(3):957-64.

Simpson CL, Patel DM, Green KJ. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol 2011;12(9):565-80.

Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G. The lin-41 RBCC gene acts in the C. elegans heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. Mol Cell 2000;5(4):659-69.

Sohn S, Schoeffski O, Prinz J, Reich K, Schubert E, Waldorf K, et al. Cost of moderate to severe plaque psoriasis in Germany: a multicenter cost-of-illness study. Dermatology 2006;212(2):137-44.

Sonkoly E, Loven J, Xu N, Meisgen F, Wei T, Brodin P, et al. MicroRNA-203 functions as a tumor suppressor in basal cell carcinoma. Oncogenesis 2012;1:e3.

Sonkoly E, Stahle M, Pivarcsi A. MicroRNAs: novel regulators in skin inflammation. Clin Exp Dermatol 2008;33(3):312-5.

Sonkoly E, Wei T, Janson PC, Saaf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One 2007;2(7):e610.

Srivastava A, Nikamo P, Lohcharoenkal W, Li D, Meisgen F, Xu Landen N, et al. MicroRNA-146a suppresses IL-17-mediated skin inflammation and is genetically associated with psoriasis. J Allergy Clin Immunol 2017;139(2):550-61.

St John AL, Rathore AP, Yap H, Ng ML, Metcalfe DD, Vasudevan SG, et al. Immune surveillance by mast cells during dengue infection promotes natural killer (NK) and

NKT-cell recruitment and viral clearance. Proc Natl Acad Sci U S A 2011;108(22):9190-5.

Streilein JW. Skin-associated lymphoid tissues (SALT): origins and functions. J Invest Dermatol 1983;80 Suppl:12s-6s.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102(43):15545-50.

Swindell WR, Johnston A, Voorhees JJ, Elder JT, Gudjonsson JE. Dissecting the psoriasis transcriptome: inflammatory- and cytokine-driven gene expression in lesions from 163 patients. BMC Genomics 2013;14:527.

Szabo SK, Hammerberg C, Yoshida Y, Bata-Csorgo Z, Cooper KD. Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. J Invest Dermatol 1998;111(6):1072-8.

Tian P, Yan L. Inhibition of MicroRNA-149-5p Induces Apoptosis of Acute Myeloid Leukemia Cell Line THP-1 by Targeting Fas Ligand (FASLG). Med Sci Monit 2016;22:5116-23.

Tiilikainen A, Lassus A, Karvonen J, Vartiainen P, Julin M. Psoriasis and HLA-Cw6. Br J Dermatol 1980;102(2):179-84.

Tonel G, Conrad C, Laggner U, Di Meglio P, Grys K, McClanahan TK, et al. Cutting edge: A critical functional role for IL-23 in psoriasis. J Immunol 2010;185(10):5688-91.

Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012;44(12):1341-8.

Tsoi LC, Stuart PE, Tian C, Gudjonsson JE, Das S, Zawistowski M, et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. Nat Commun 2017;8:15382.

Turchinovich A, Samatov TR, Tonevitsky AG, Burwinkel B. Circulating miRNAs: cell-cell communication function? Front Genet 2013;4:119.

Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta 2014;1843(11):2563-82.

Urgard E, Lorents A, Klaas M, Padari K, Viil J, Runnel T, et al. Pre-administration of PepFect6-microRNA-146a nanocomplexes inhibits inflammatory responses in keratinocytes and in a mouse model of irritant contact dermatitis. J Control Release 2016;235:195-204.

Uto-Konomi A, Miyauchi K, Ozaki N, Motomura Y, Suzuki Y, Yoshimura A, et al. Dysregulation of suppressor of cytokine signaling 3 in keratinocytes causes skin inflammation mediated by interleukin-20 receptor-related cytokines. PLoS One 2012;7(7):e40343.

Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 1993;101(5):701-5.

van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol 2009;182(9):5836-45.

Wang Y, Zheng X, Zhang Z, Zhou J, Zhao G, Yang J, et al. MicroRNA-149 inhibits proliferation and cell cycle progression through the targeting of ZBTB2 in human gastric cancer. PLoS One 2012;7(10):e41693.

Watson W, Cann HM, Farber EM, Nall ML. The genetics of psoriasis. Arch Dermatol 1972;105(2):197-207.

Veal CD, Clough RL, Barber RC, Mason S, Tillman D, Ferry B, et al. Identification of a novel psoriasis susceptibility locus at 1p and evidence of epistasis between PSORS1 and candidate loci. J Med Genet 2001;38(1):7-13.

Welsch K, Holstein J, Laurence A, Ghoreschi K. Targeting JAK/STAT signalling in inflammatory skin diseases with small molecule inhibitors. Eur J Immunol 2017;47(7):1096-107.

Villanova F, Flutter B, Tosi I, Grys K, Sreeneebus H, Perera GK, et al. Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. J Invest Dermatol 2014;134(4):984-91.

Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 2007;8(9):950-7.

Wolk K, Witte E, Wallace E, Docke WD, Kunz S, Asadullah K, et al. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol 2006;36(5):1309-23.

Xu G, Zhang Z, Xing Y, Wei J, Ge Z, Liu X, et al. MicroRNA-149 negatively regulates TLR-triggered inflammatory response in macrophages by targeting MyD88. J Cell Biochem 2014;115(5):919-27.

Xu N, Brodin P, Wei T, Meisgen F, Eidsmo L, Nagy N, et al. MiR-125b, a microRNA downregulated in psoriasis, modulates keratinocyte proliferation by targeting FGFR2. J Invest Dermatol 2011;131(7):1521-9.

Xu N, Meisgen F, Butler LM, Han G, Wang XJ, Soderberg-Naucler C, et al. MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40. J Immunol 2013;190(2):678-88.

Yao Z, Spriggs MK, Derry JM, Strockbine L, Park LS, VandenBos T, et al. Molecular characterization of the human interleukin (IL)-17 receptor. Cytokine 1997;9(11):794-800.

Yi R, Fuchs E. MicroRNA-mediated control in the skin. Cell Death Differ 2010;17(2):229-35.

Yi R, O'Carroll D, Pasolli HA, Zhang Z, Dietrich FS, Tarakhovsky A, et al. Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. Nat Genet 2006;38(3):356-62.

Yi R, Pasolli HA, Landthaler M, Hafner M, Ojo T, Sheridan R, et al. DGCR8-dependent microRNA biogenesis is essential for skin development. Proc Natl Acad Sci U S A 2009;106(2):498-502.

Yi R, Poy MN, Stoffel M, Fuchs E. A skin microRNA promotes differentiation by repressing 'stemness'. Nature 2008;452(7184):225-9.

Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 2003;17(24):3011-6.

Yost J, Gudjonsson JE. The role of TNF inhibitors in psoriasis therapy: new implications for associated comorbidities. F1000 Med Rep 2009;1.

Yu AP, Tang J, Xie J, Wu EQ, Gupta SR, Bao Y, et al. Economic burden of psoriasis compared to the general population and stratified by disease severity. Curr Med Res Opin 2009;25(10):2429-38.

Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Johnson-Huang LM, Nograles KE, White TR, et al. Identification of TNF-related apoptosis-inducing ligand and other molecules that distinguish inflammatory from resident dendritic cells in patients with psoriasis. J Allergy Clin Immunol 2010;125(6):1261-8 e9.

Zenewicz LA, Flavell RA. Recent advances in IL-22 biology. Int Immunol 2011;23(3):159-63.

Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, et al. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature 2005;437(7057):369-75.

Zhang H, Kolb FA, Brondani V, Billy E, Filipowicz W. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. EMBO J 2002;21(21):5875-85.

Zhang H, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W. Single processing center models for human Dicer and bacterial RNase III. Cell 2004;118(1):57-68.

Zhang W, Yi X, Guo S, Shi Q, Wei C, Li X, et al. A single-nucleotide polymorphism of miR-146a and psoriasis: an association and functional study. J Cell Mol Med 2014;18(11):2225-34.

Zhang XJ, Huang W, Yang S, Sun LD, Zhang FY, Zhu QX, et al. Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. Nat Genet 2009;41(2):205-10.

Zhao JL, Rao DS, Boldin MP, Taganov KD, O'Connell RM, Baltimore D. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci U S A 2011;108(22):9184-9.

Zibert JR, Lovendorf MB, Litman T, Olsen J, Kaczkowski B, Skov L. MicroRNAs and potential target interactions in psoriasis. J Dermatol Sci 2010;58(3):177-85.

Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, et al. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature 2012;484(7395):514-8.

Zouboulis CC. Human skin: an independent peripheral endocrine organ. Horm Res 2000;54(5-6):230-42.

"Whatever deity may guide my life, dear lord don't let me die tonight.

But if I shall before I wake, I'd accept my fate."

"Jay-Z"